PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

CHARLES MANNING CHILD AND WARDER C. ALLEE
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REGENERATION IN THE POLYCHAETE CLYMENELLA TORQUATA. II. EFFECT OF LEVEL OF CUT ON TYPE OF NEW STRUCTURES IN POSTERIOR REGENERATION.

LEONARD P. SAYLES
Marine Biological Laboratory and College of the City of New York

(Fifteen Figures)

THE main points concerning the external features of posterior regeneration in *Clymenella torquata*, Leidy, after the removal of 6 segments, have already been described (Sayles, 1932). Under these conditions worms which survive the operation replace exactly the number and arrangement of segments which were lost. It is the purpose of this paper to report the response of this species in posterior regeneration when cut at or near any one of the 21 intersegmental regions present in this species.

The experimental part of this work was carried on at Woods Hole during the four summers of 1928–31, inclusive.

MATERIALS AND METHODS

In the above-mentioned paper on this species it was reported that many worms are found to be regenerating under natural conditions. During the summer of 1931, 734 individuals were collected. Of these, 188 (25.6 per cent) were regenerating posteriorly and 10 (1.4 per cent) anteriorly. Most of the worms were dug on Nonamesset Flats where only 90 (18.4 per cent) were regenerating. The others came from Northwest Gutter between Naushon and Uncatena Islands and from Sheep's Pen and Lackey's Bay on Nonamesset. Table I gives a summary of anterior and posterior regenerates found during 1931.

Perhaps the most interesting point about these data is that one individual was found which had formed 12 new posterior segments; i.e., it had regenerated at the tenth segment. This is the first case—among 1,354 worms—of posterior regeneration under natural conditions at a level anterior to the twelfth segment. In this case a new bud had been formed with 12 new segments and a complete anal

TABLE I

REGENERATION UNDER NATURAL CONDITIONS

	Number of New Segments										Total Regenerates	Non-Regenerates
	1	2	3	4	5	6	7	8	9	12		
Posterior regeneration	0	39	67	37	17	15	7	4	1	1	188	546
Anterior regeneration	0	5	3	1	0	1	0	0	0	0	10	724

collar with cirri. As in previous collections, no specimens were found during 1931 which had been regenerating anteriorly at a level posterior to the sixth segment.

During the entire four years only one specimen (Fig. 1) has been found under natural conditions which showed any indications of bifurcation. Since this was at the posterior end it may be well to describe it at this time. All segments except the last were of the usual appearance. The anterior portion of this segment was of usual size in proportion to the twenty-first segment. Of the two posterior parts, the larger was of smaller size than is usually the case. Consequently there was a ledge-like lateral portion of the main part of the segment. This was on the right side. Set on this and directed posteriorly was a small outgrowth with an anal collar and 8 cirri.

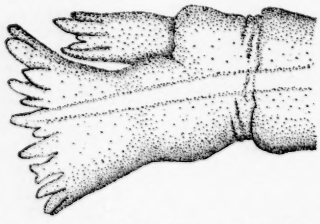

FIG. 1.—Ventral view of double anal segment occurring in nature. ×12.5.

The fact that the larger part was of reduced size to compensate for

the presence of the smaller leads to the conclusion that both were formed together. That is, this was probably a case of bifurcation in development rather than of lateral outgrowth of the smaller bud after the completion of a typical posterior end. Since there were no distinct color differences to indicate that regeneration had occurred, it may be that the doubling took place in the course of embryonic development. If not, then it was produced in regeneration a considerable time before this specimen was collected.

For these experiments all worms were cut on a paraffin block with a razor blade. The pieces were then placed in large dishes containing about $\frac{3}{4}$ inch of clean sea water. The water was changed about twelve hours after the operation and each morning thereafter.

In all cases the cut was made as near as possible to the level of the intersegmental septum. In the anterior part of the body this septum lies near the parapodia of the segment posterior to it. In the posterior region the septum is immediately posterior to the parapodia of the segment anterior to it.

Histological preparations of this material have not as yet been studied. At the present time, however, some of the regenerates described below are being prepared for this purpose.

WOUND CLOSURE

At the first to third segments, wound closure is usually complete within two hours. In most cases at the fourth to sixth a bulb of gut is formed which disappears and permits completion of wound closure within twenty-four hours. Posterior to the sixth segment bulbs are usually formed and may persist for three or four days. Most pieces in which wound closure has not been completed by the fifth day, however, do not survive very much beyond that time. In the case of the smaller pieces, disintegration commonly begins in the wound region within a few days after wound closure.

VIABILITY

The percentage of pieces surviving the operation of cutting is, to a certain degree, proportional to the size of these pieces. The data for percentage of survivors at the end of a ten-day period at the different levels in these experiments are as follows: first and second segments, 0; third, 18.1; fourth, 13.3; fifth, 10.8; sixth, 23.4; seventh,

31.1; eighth, 42.4; ninth, 45.8; tenth, 72.2; eleventh, 61.8; twelfth, 73.9; thirteenth, 83.3; fourteenth, 84.6; fifteenth to twenty-first, 85 or over.

POSTERIOR REGENERATION

All of the individuals regenerating at each level will be considered together. It is to be understood, however, that every group is made up of pieces from experiments of two or more summers. In fact, in most cases pieces are included from each of the four summers during which these experiments have been carried on.

Anterior pieces of *one or two segments* died within one week after cutting. None of these showed any evidence of new tissue.

Occasionally a piece of *three to five segments* lived as long as three weeks. Even in those surviving that length of time, no new tissue was visible.

Six-segment pieces.—Seventy-seven were cut and 13 were alive after twenty days. One of these had a bulb of gut at the wound region, the others had completed wound closure. Five of the survivors had a small amount of light-colored tissue at the closed cut-surface. Ten days later only these five individuals were alive and they had not changed appreciably in appearance. These were lost a few days later without further changes having occurred.

Seven-segment pieces.—One hundred and fifty-one worms were cut and 24 of these were alive after sixteen days. Of these, 16 were without new tissue; 5 had traces of new tissue similar to those described above; and 3 had very short buds. One of the latter is shown in Figure 2. By the thirtieth day there were but 8 survivors: 1 with no new tissue and 7 with possible traces of such.

Eight-segment pieces.—Of 132 cut, 56 lived at least ten days. At this time 30 were without new tissue, 7 showed possible traces of new tissue, 7 had short, somewhat irregular buds, 7 had slender buds with an anal cone protruding beyond the collar-anlage (Fig. 3), and each of the remaining 5 had a short bud, the posterior part of which was open on the dorsal side (see Fig. 14).

By the sixteenth day the 7 with slender buds and 2 of those with buds open dorsally had replaced the part lost. That is, these 9 had formed 11 setigerous plus 3 asetigerous segments. The anal segment bore the usual collar with cirri. The number of the latter on each

collar, however, was considerably less than the average for uninjured worms. These regenerates had 8, 9, 10, 10, 10, 11, 12, 12, and

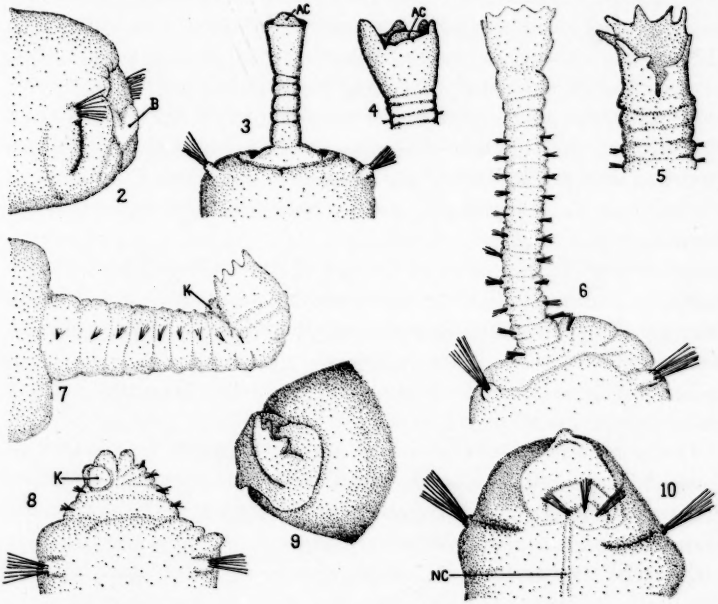

FIG. 2.—Lateral view of posterior regenerate at seventh segment after sixteen days. *B*, bud of new tissue. ×17.

FIG. 3.—Dorsal view of regenerate at eighth segment after eleven days. *AC*, anal cone. ×10.

FIG. 4.—Dorsal view of last four segments of a bud formed at the eighth segment. *AC*, anal cone. Sixteen days. ×24.

FIG. 5.—Dorsal view of last five segments of a bud formed at the eighth segment after fourteen days. ×17.

FIG. 6.—Dorsal view of bud formed at eighth segment after eighteen days. ×12.5.

FIG. 7.—Lateral view of regenerate at eighth segment after fifteen days. *K*, knob of tissue protruding from dorsal opening in bud. ×12.5.

FIG. 8.—Dorsal view of regenerate at eighth segment after nineteen days. *K*, knob of tissue growing dorsally from posterior part of bud. ×10.

FIG. 9.—Lateral view of bud formed at eighth segment after fourteen days. ×17.

FIG. 10.—Ventral view of new tissue formed at eighth segment after twenty-eight days. *NC*, nerve cord of old part. ×17.

13 cirri, respectively, to give an average of about 10.6. For comparison, the corresponding data for 50 intact worms taken at random

were obtained. They are: 2 with 19; 9 with 20; 10 with 21; 17 with 22; 6 with 23; 4 with 24; 2 with 26; average 21.7.

It is apparent that on each collar there were present about half as many cirri as on uninjured specimens. In one of these specimens (Fig. 4) the cirri—8 in number—were short and of large diameter; twelve days later this individual had 19 cirri. In no other case, however, was there an increase in the number of cirri during this additional time. On 6 of the regenerates the dorsal side of the collar region was shorter than the ventral and in 5 there was a mid-ventral cirrus longer than any of the others. Such a mid-ventral cirrus appears in Figure 15.

Another of the survivors at the end of sixteen days had formed a complete bud except for the fact that the collar was open dorsally (Fig. 5). Two others had formed complete but quite slender buds as in Figure 6.

Ten worms had produced no new tissue at this time and 2 others had developed possible traces of new material.

The remaining 5 survivors had formed irregular buds. One of these (Fig. 7) had produced 9 new setigerous segments followed by an asetigerous region which was tipped dorsally. The very short dorsal region associated with this tipping was open into the posterior part of the digestive tract. There was no separate anus but the terminal membrane was slightly notched at its margin of contact with the dorsal opening. There was present a U-shaped collar region apparently corresponding to the ventral and lateral portions of a normal collar. On this there were 9 slender anal cirri. This worm died after seventeen days.

Another of these irregular buds (Fig. 8) was somewhat conical in shape and bore 2 notopodia on the right side and 4 on the left. In addition, there was a small dorsal projection near the posterior tip of the bud. The condition of this worm was not appreciably changed after thirty-one days.

The third of these worms bore a very slender bud with 1 notopodium, on the right side. This also had a small portion growing out dorsally near the posterior tip of the bud. At the time when this piece died, after twenty-three days of regeneration, there was some evidence of a branching at the extreme posterior tip.

The fourth bud had a short portion turned dorsally and apparently consisting of merely the anal segment, the dorsal part of which was missing (Fig. 9). On the collar region there were 5 short, thick cirri. This part of the bud came out from a fairly flat region of new tissue which covered much of the terminal portion of the old part. From the ventral part of this flat region, near the end of the old nerve cord, a small point of new tissue was growing out. Unfortunately this worm did not live beyond this time. Consequently this structure—possibly double—could not be followed further.

The last of these irregular buds is shown in Figure 10. The old part had contracted in such a way that the dorsal part of the wound region overhung the ventral part to some extent. The new tissue which formed consisted of a rather flat portion from the dorsal part of which a point of growth projected. On the ventral part, near the old nerve cord, there was a rounded elevation on which there was a fan of 3 notopodia. At the end of twenty-eight days this new tissue had not changed its appearance and at this time the worm was drawn and preserved.

Nine-segment pieces.—At the end of ten days 33 out of 72 pieces were alive. Of these, 9 had weakly segmented buds with short collars as in Figure 3. Eleven were slightly more advanced with short cirri and more distinct segmentation. One had formed a bud open dorsally (see Fig. 14). Two were without new, and 5 showed possible traces of new, tissue. The remaining 5 had formed buds of irregular structure.

Eight days later the condition of these pieces was as follows: Of the 20 with buds of good shape, 4 had died, and 16 were now complete with 10 new setigerous and 3 asetigerous segments. The average number of cirri present on the collars of these was 17.3, with the largest number 22. In most cases the mid-ventral cirrus was longer than the others.

The bud which was open dorsally had become complete but with the anal segment as in Figure 5. One of those with no new tissue had died, the other was still without new material and remained in this condition until it was discarded, twenty-two days after the cut. Of the 5 which showed possible traces of new substance at ten days, 4 had died and the other had an early bud of the type of Figure 3.

Two of those with irregular buds had died and the 3 remaining had very short buds consisting of not much more than the region of the anal segment incomplete dorsally. Two of these are shown in Figures 11 and 12.

Ten-segment pieces.—Out of 36 cut, 26 were alive after ten days and 17 after twenty days. At ten days there were 10 with weakly segmented buds with collars and small cirri—slightly more advanced than Figure 3. After twenty days 3 of these were dead and 7 were complete with 9 setigerous plus 3 asetigerous segments.

At ten days, 12 others had weakly segmented buds with the beginnings of collars but open dorsally as shown in Figure 14. After twenty days, 4 of the latter were dead; 3 were complete; 3 were complete except for the fact that the collar had a cleft along the mid-dorsal line (Fig. 15); 1 had a well-formed bud but consisting of only 5 setigerous plus 3 asetigerous segments and with its anal collar open dorsally; the remaining 1 had formed a bud of irregular shape, perhaps associated with the loss of a portion of the previously formed bud.

At ten days, 3 other individuals were without new growth. Of these, 2 were dead after twenty days. The other had not regenerated when it was finally discarded four weeks after the original cutting.

At ten days, 1 individual had formed a bud of good shape and complete with 12 segments present.

Of the 11 individuals complete after twenty days, 4 had a long mid-ventral cirrus and 2 had a collar slightly shorter on the dorsal side. The average number of cirri present on these buds was 18.8, with the largest number 21.

Eleven-segment pieces.—Thirty-four pieces were cut; 21 survived for ten days. At this time 8 were slightly more advanced than Figure 3. Ten had buds open dorsally as in Figure 14. Two were without new tissue and 1 had a possible trace. After twenty days 20 specimens were still alive and, of these, 10 were complete with 11 new segments. The average number of cirri present on these was 26.9. One individual had formed a collar with 30 cirri. At this same time 7 others were complete except for the fact that the dorsal portion of the anal collar was short or missing. Of the remaining 3, 2 were without new tissue and 1 had a slender unsegmented bud of

the type of Figure 3. Eventually all except the 2 without new tissue formed complete buds. Of these, 3 had anal collars with mid-dorsal clefts as in Figure 15.

Twelve-segment pieces.—Of 23 pieces cut, 17 survived ten days. Of these, 14 were complete and 3 had well-formed tail buds with rather indistinct segmentation and collars slightly shorter dorsally. After

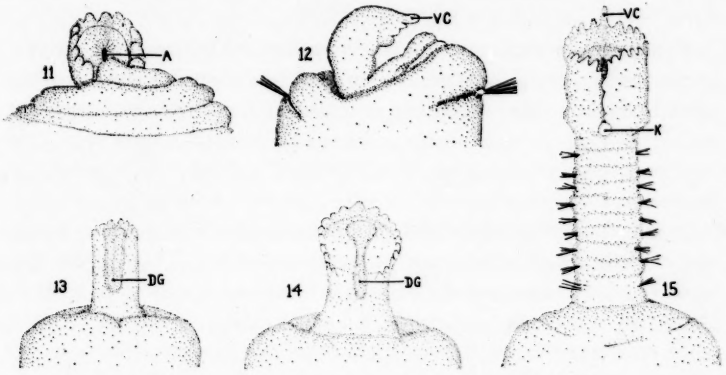

FIG. 11.—Dorsal view of bud formed at ninth segment after twelve days. *A*, anus. ×12.5.

FIG. 12.—Ventral view of posterior regenerate at ninth segment after twelve days. The rotated condition of the bud was natural. *VC*, ventral cirrus. ×12.5.

FIG. 13.—Dorsal view of regenerate at tenth segment after six days. *DG*, dorsal groove. ×10.

FIG. 14.—Dorsal view of same bud as in Figure 13. After nine days. *GD*, dorsal groove. ×12.5.

FIG. 15.—Dorsal view of same individual as in Figures 13 and 14. After fifteen days. *VC*, ventral cirrus; *K*, knob of tissue extending dorsally through posterior end of slit in anal collar. ×12.5.

twenty days all of these were complete. The average number of anal cirri present was 28.8. The greatest number found on any collar was 38.

Thirteen-segment and longer pieces.—Pieces cut to regenerate posteriorly at these levels survived in over 80 per cent of the cases. In fact, it is doubtful if many of the deaths in this particular material were the direct result of the injury. In every instance survivors eventually formed complete buds consisting of the number of seg-

ments lost. In a number of cases, especially at the thirteenth to fifteenth segments, the anal collar was at first shorter on the dorsal side. Within fourteen to twenty days after the cut, however, it was of typical shape. At the thirteenth segment the average number of anal cirri was again large—27.4—but at more posterior levels the average was between 20 and 25.

DISCUSSION

Clymenella torquata is a species characterized by the presence of 22 segments. In most annelids it is difficult to determine when regeneration ends and when ordinary increase in the number of segments accompanying growth begins. Here, in *Clymenella*, however, new-segment production must be attributed entirely to regenerative processes.

In this form regeneration is complete, or practically so, in all survivors at the twelfth and more posterior segments. Then, as one goes forward, there is a gradual decrease in the number of worms with the ability to replace completely parts lost. Finally, at the seventh segment none are able to do so. Within this mid-body region, however, there is no real uniformity in the response of all individuals cut at the same level. At the end of sixteen days, for example, 41.4 per cent of the survivors at the eighth segment had formed practically complete buds, yet an equal number had produced little or no new tissue. At the ninth segment, 77.3 per cent had formed virtually complete buds; at the tenth, 82.3 per cent; at the eleventh, 90 per cent; at the twelfth and more posterior levels, practically 100 per cent. At each of the levels anterior to the twelfth there was also at least one piece without evidence of new material. It was not possible to discover any marked morphological differences between non-regenerating pieces and those forming complete buds. Some in each group were small and light-colored, others were large and dark-colored. Individuals collected at the same time and in the same place and treated, as far as possible, in the same manner were found in both categories.

At the level of the eighth segment, the anteriormost to form more than very short, unsegmented buds, 12 of the 29 survivors had formed practically complete buds. In 6 the only defect externally discernible was a short dorsal portion of the anal collar. In another

the collar was completely missing in the mid-dorsal region. Aside from these minor defects, the 12 new buds at this level differed from those at the twelfth, for example, mainly in that they were more slender and, of course, of greater length as a result of the 4 additional segments present. The actual volume of the bud, however, is considerably greater at the more posterior level. This is clearly shown by a comparison of 1 regenerating at the eighth segment (Fig. 6) and 1 at the tenth (Fig. 15). The total amount of tissue at the latter level is more than 50 per cent greater than that at the former. It does not seem unreasonable to attribute the slender condition of the longer bud to the fact that at the level in question the piece is not able to produce as much material as at a more posterior level.

Similarly, less complete buds, down even to those consisting of the merest traces of new material, should be attributed to scarcity of material rather to a lessened potency of some "differentiating factor." Whatever this factor may be, it appears to exert its influence as thoroughly on the material available at the eighth as at the fifteenth segment. There is, apparently within certain limits, an attempt to return the individual to the fixed number of metameres. In some cases, however, as at the level of the eighth segment, the amount of material provided may be inadequate.

In only 2 cases was a considerable amount of material formed and yet not organized into the same number of segments as was lost. One bud, at the eighth segment (Fig. 7), had only 9 setigerous segments. In this case, too, the dorsal part of the last 3 (asetigerous) segments was much shortened, with the result that this region was turned toward the dorsal side. Ventrally, however, there was good evidence of the presence of the three segments. In the other bud, at the tenth segment, only 5 setigerous segments were formed but the 3 asetigerous were clearly present. The anal segment was open dorsally but it was not tipped. In these 2 exceptional instances, then, there are setigerous segments missing—2 in the former, 4 in the latter. The condition of these buds may possibly be considered as due to a slight deficiency in the amount of material necessary to form the entire number of segments required to replace the part lost.

Most of the remaining pieces which form incomplete new buds produce relatively little new tissue. In such cases the bud consists of

little more than the anal segment with its collar and cirri. The dorsal part of this type of bud is considerably shortened and the collar and cirri are missing dorsally. In the case of ordinary regeneration at posterior levels, the anal segment is the first to be formed, at a time when the bud is very small. Here, then, there is insufficient material to go beyond this stage in regenerative development.

When a very small amount of new tissue is formed, it may develop into a short unsegmented bud without trace of anal collar or cirri, as in several pieces at the seventh segment. Aside from 1 case, in which a distinctly atypical fan of 3 notopodia was formed (Fig. 10), all buds, at whatever level developed, differed from the normal only in an orderly reduction of some part or parts. As just mentioned, this reduction, of course, may extend so far that only an anal segment or even a completely unorganized bud is produced.

Examining the bud-forming activities of *Clymenella* from this point of view, one may conclude that the responses of different pieces at various levels are dependent in each case upon the amount of material which the particular piece is able to supply at the wound region. As far as posterior regeneration is concerned, 3 regions may be distinguished in *Clymenella*. The anteriormost, including the first 6 segments, is capable of little, if any, new-tissue formation. In the most posterior region, involving the twelfth-segment level and those more posterior, all survivors are able to replace completely the parts lost. In the intermediate region there is a gradual increase, from anterior toward posterior, in the average amount of material which may be produced. On the other hand, the organizing factors, whatever they may be, vary from one level to another only to the extent of replacing completely the part lost from each. For an explanation of the various states of incompletion of the buds, one must seek, therefore, a factor or factors exerting an influence on the amount of material which the pieces in question may produce. To conclude that the factors involved belong in one category or another would be inadvisable at this time. Before taking that step one must examine carefully the effects, on both intact and regenerating worms, of changes in various conditions both external and internal.

One may also seek an explanation for the fact that the dorsal region is the first affected by an inadequate supply of material. In

very small buds (Fig. 13), on the way to forming quite complete parts, the dorsal side may be open for a considerable distance. Obviously, then, it is a question of the relation of the amount of material to the particular stage reached in the organization of the bud. As increase in size of the bud continues, the dorsal groove decreases in extent until ultimately it may include only the collar region.

In the case of other polychaetes, there is some disagreement concerning the exact source of new material for regeneration. Langhammer (1928) does not suggest this source. Okada (1929) says new parts are formed from old much as in oligochaetes. Michel (1898), Nusbaum (1905 and 1908), and Iwanow (1906 and 1928) all believe it to be of ectodermal origin. On the other hand, Probst (1931) and Faulkner (1932) believe that a proliferation of interneural neoblasts occurs, from which all the tissues of the newly forming bud are derived. All of these workers, however, are in agreement that there is formed a ventral cell-mass which becomes the immediate source of much or all of the new bud. In *Clymenella*, therefore, it is fairly probable that such a ventral cell mass is produced. By an organization of this the new bud would be formed. As a result, when insufficient material is provided, the dorsal region, which is farthest from the source point, is the first affected. Further information concerning this particular point may perhaps be obtained through a study, now being made, of the histology of regeneration in *Clymenella*.

These experiments suggest the possibility that the posterior tip is established first and, then, under its influence, the rest of the bud is organized. In apparent contradiction to this idea, are buds in which a few distinct setigerous segments are formed adjacent to the old part and followed by a more or less irregular region. These have no trace of an anal segment. In these two or three cases, however, it is probable that the posterior part of the bud was lost in some manner, especially since not infrequently a bud is found with a mid-portion more slender than either base or posterior part. If these irregular buds do arise in this manner, then the anal segment could still have exerted its influence on their organization. In this connection it should also be noted that at the seventh segment, where no collar anlage appears, the bud is without segmentation.

That there can be some organization of the new material in the

absence of the anal segment or of segmentation is demonstrated in the case of the fan of 3 notopodia (see Fig. 10). Here there is formed an extremely small amount of new material, perhaps the least in which there was any evidence of organization. This is the only distinctly atypical structure observed in the posterior regeneration of this species.

As already mentioned, there is considerable variation in the number of anal cirri formed. Frequently, especially in the mid-body region, the mid-ventral cirrus is distinctly longer than the others. Also, in a number of cases, the collar was rather short and the anus raised up on the tip of a cone which might even extend beyond the limits of the collar. In this connection it is interesting to note that in ordinary adults of another species of the same family—*Praxillella praetermissa* of the Maldanidae—according to McIntosh (1915, III, Part I, 329) "the anal funnel appears to vary considerably in regard to the number and the shape of the cirri. . . . . The elongated cirrus is in the mid-ventral line. The anal cup is comparatively shallow, with the anus often on a slight cone in the center." In *Praxillella* there is also considerable variation in the number of cirri. The extremes mentioned by McIntosh are 16 and 37.

It would seem, then, that in posterior regeneration, particularly in the mid-body region, *Clymenella* frequently forms an anal segment, collar, and cirri somewhat different from those ordinarily found in this species. In fact, at times it is more like the perhaps less complex plan of these structures in other species of the same family.

There is also a fair parallel between the percentage of survivals and the level of the cut. The data for this are given on pages 3–4. There are probably several factors involved in the high mortality at anterior levels as compared with posterior parts of the body. Many anterior pieces probably die because of their small size. Moreover, at some of these levels, a slow wound closure increases the chances of an infection. Posterior to the ninth segment wound closure is more rapid. In this connection it should be noted that autotomy apparently does not occur anterior to the tenth segment. In the region of the tenth to twelfth segments it may be experimentally produced, though with some difficulty. Posterior to the twelfth it occurs very readily. Hence worms are equipped for more rapid wound closure and reduction in the chances of infection at these levels.

Under natural conditions only one specimen has been found regenerating posteriorly at a level anterior to the twelfth segment; namely, at the tenth. Following experimental cuts, however, complete buds may be formed in the majority of cases, even at the ninth segment. There are probably at least two factors involved in this difference. First, a great many of the regenerates found in nature are the result of autotomy which is more or less limited to these posterior levels. Second, and probably of less significance, the number of pieces produced in nature by accidental breaks anterior to the twelfth segment is probably few. Most of such individuals probably die soon after injury.

SUMMARY

1. Anterior pieces of one to five segments do not regenerate beyond the closing of the wound. A few of six segments appear to produce a little new tissue at the wound region.

2. On the other hand, anterior pieces cut at the twelfth segment and more posterior levels replace completely the parts lost. Occasionally buds appear which at first have anal collars short in the dorsal region. In every case these eventually attain the usual form.

3. In the mid-body region there is a gradual transition from short, apparently unorganized buds at the seventh segment to a high percentage of complete buds at the eleventh segment. There is no real uniformity in the response of all individuals cut at the same level. The percentage of practically complete individuals after sixteen days' regeneration at the mid-region levels are as follows: at the eighth segment, 41.4; at the ninth, 77.3; at the tenth, 82.3; at the eleventh, 90; at the twelfth and more posterior levels, practically 100.

4. The formation of incomplete buds should be attributed to the inability of the pieces in question to form a sufficient amount of new material to produce a complete bud. Whatever the "organizing factor" may be, it seems to exert its influence as thoroughly on the material available at the eighth segment as on that at the fifteenth or more posterior segments.

5. These experiments suggest the possibility that the posterior tip is established first and, then, under its influence, the rest of the bud is organized.

6. There is a fair parallel between the percentage of survivals and the level of the cut.

7. From the point of view of anal cirri there are three zones: (*a*) the eighth to tenth segment region in which the average number produced is below the usual number (about 22 on the average) found on intact individuals; (*b*) the eleventh to thirteenth segment region with an average about 5 above the usual number and the greatest number in these experiments 38; (*c*) the fourteenth to twenty-first segment region in which approximately the usual number is replaced.

8. None of these posterior regenerates produced heteromorphic heads. No unquestionable case of a double structure or a branching bud appeared.

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THE EFFECT OF CHLORETONE ON THE OXYGEN CONSUMPTION OF AMPHIBIAN LARVAE (RANA CLAMITANS)[1]

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NARCOTIC substances have long been known to exert a marked effect on the irritability and respiratory exchange of organisms. Perhaps the first reliable experiments were those of Geppert (1889) who found that the oxygen consumption of mammals was increased during the first stages of cyanide poisoning although it later fell, markedly, when all the toxic symptoms had developed. He was led, therefore, to the conclusion that cyanide poisoning directly affected the oxygen consumption of the individual cells. The respiratory exchange of aquatic animals has been measured in the presence of various narcotic substances. Burge (1924), using chloroform, ether, nitrous oxide, and ethylene on *Paramecium* and *Colpoda*, found that chloroform produced the greatest decrease in oxygen consumption; nitrous oxide and ethylene having but slight effect on *Colpoda* and no effect on *Paramecium*. Experiments by Hyman (1916) on the marine sponge *Suberites* indicated that exposure to dilute solutions of KCN increased the rate of oxygen consumption, whereas high concentrations decreased the rate to a marked degree. Hyman (1919*b*) reported that with KCN the depression of oxygen consumption in *Planaria* depended upon the concentration used; since a 0.002 molar solution gave a depression of 80–90 per cent, while a 0.00002 molar solution resulted in a depression of but 5–15 per cent. Allen (1919), working with *Planaria*, found that dilute solutions of KCN did not stimulate oxygen consumption. The results obtained by Buchanan (1926) showed that the oxygen consumption of *Planaria* fell to a constant low-level during four hours' exposure to a .025 molar solution of KCN. After removal of the

[1] This investigation was carried out at the suggestion of Dr. O. M. Helff, to whom the writer wishes to express his gratitude for suggestions and aid during the course of the work and in the preparation of the manuscript.

narcotic, however, oxygen consumption rose above normal in six hours. He also found that ether, in concentrations of 0.25, 0.5, and 0.75 per cent, stimulated oxygen consumption.

Loeb and Wasteneys (1911) reported that cyanide decreased the rate of oxygen consumption in sea-urchin eggs. Hyman (1919*a*) measured the oxygen consumption of some coelenterates and annelids, including *Pennaria*, *Metridium*, *Nereis*, *Chaetopterus*, *Arenicola*, and two species of aquatic Oligochaetes, in the presence of various concentrations of KCN and found that in all cases there was a reversible depression.

Irwin (1918) tested the effect of ether on CO_2 production in several organisms including tadpoles, frog eggs, *Fundulus* embryos, and the aquatic insect *Dineutes assimilis*. This author obtained reversible and irreversible depressions depending on the concentration used. Helff (1926) etherized *Rana pipiens* tadpoles, using a 2 per cent concentration, and obtained an increase in oxygen consumption of 19 per cent. A second etherization raised respiration 27 per cent or an increase of 8 per cent above the first exposure to ether. Narins (1930), testing the effect of various concentrations of chloroform on the oxygen consumption of *Rana clamitans* tadpoles, found that, with solutions ranging from 0.01 to 0.1 per cent, marked reversible depressions were produced. Lower concentrations tested did not show any measurable effect as regards stimulating or depressing oxygen consumption. The evidence presented in the foregoing abbreviated survey indicates that dilute solutions of depressing substances generally stimulate respiratory exchange and, secondly, that more concentrated solutions, within certain limits, cause marked depressions of respiratory exchange which are reversible.

The effects produced by chloretone on the irritability and respiratory exchange of aquatic animals have been reported in comparatively few cases. Buchanan (1922) pointed out that, due to its depressing effect, chloretone prevented the usual rise in oxygen consumption that follows sectioning *Planaria*. Pieces of *Planaria* immersed in dilute solutions over long periods of time were stimulated, the rate of oxygen consumption rising continuously. The same author (1923) also found a stimulating effect on the respiration of whole animals upon extended exposure to dilute solutions of chloretone.

Carlson (1906) reported that the heart beat of *Limulus* showed a faster rhythm in weak solutions of this anesthetic. Harrison (1904) made an experimental study of the relation of the nervous system to the developing musculature in the embryo of the frog, narcotizing the frog embryos (*Rana virescens and R. palustris*) with chloretone. He was of the opinion that the general retardation of the development of the narcotized embryos results from general disturbances in metabolism of the cells, possibly in their diminished power of oxidation. No oxygen consumption tests were made, however. Mathews and Detwiler (1926), in their study on the reflex activity of *Amblystoma* embryos reared in chloretone solutions, obtained similar results and reiterated Harrison's opinion in explaining the general retardation on development and reflex activity that occurred.

The results obtained in the cases as cited above indicate that chloretone probably influences respiration of aquatic animals in a manner similar to other narcotic substances. As far as the writer is aware, relatively little, if any work dealing with the effect of chloretone on the respiratory exchange of amphibian larvae has been done. Furthermore, in view of the fact that chloretone is used in various studies on aquatic animals and that the results of such observations in certain cases have been attributed indirectly to disturbances in cellular oxidation, it was thought worth while to determine the exact nature of the effect of this anesthetic on the respiratory exchange of tadpoles. This paper, therefore, presents a quantitative study of the action of various concentrations of chloretone on the oxygen consumption of the tadpole, *Rana clamitans*.

MATERIAL AND METHODS

The animals used in all tests were first-year larvae of *Rana clamitans*. They were obtained from Cincinnati, Ohio, in shipments of 250 every three weeks during the period of December, 1930—March, 1931. The tadpoles were assorted and specimens of approximately the same weight and size were kept in separate tanks. These animals ranged in weight from 1.9 to 3.2 grams and their body length varied from 60 to 70 mm. Their hind limbs, in most cases, were as yet undeveloped, being represented by small $\frac{1}{2}$-millimeter buds.

The method employed for determining oxygen consumption was

based on Birge and Juday's (1911) modification of the Winkler iodometric method for measuring dissolved oxygen in water. The water used in the oxygen consumption tests was prepared by shaking tap water with hot water in the ratio of 3:1. This water was set aside for twenty-four hours before being utilized in the tests. Water prepared in this manner was tested for oxygen content over a period of several hours and only slight variations in oxygen tension were found to occur. The concentrations of chloretone solution used in this study were prepared by weighing the chloretone accurately and then dissolving the required weight in a definite amount of tap water. High concentrations of the solution, when kept in rubber-stoppered bottles, were found to keep their strengths for several months. Lower concentrations employed in these tests were prepared by dilution of solutions of high concentration. Hastening solution of chloretone, which is difficult of solution in cold water, by heating was found to be impractical since it gave rise to error in strength of the anesthetic.

Animals were tested for four consecutive hours, samples of water being drawn at the end of each hour for oxygen determination. The respiration of the experimental animals was expressed in terms of total cubic centimeters of oxygen consumed irrespective of body weight. The temperature at which the tests were made was $25 \pm 1^{\circ}$ C. More detailed accounts of the procedures employed in the different types of tests will be given later on under their respective headings.

NORMAL VARIATION IN OXYGEN CONSUMPTION

Preliminary to determining the action of various concentrations of chloretone on oxygen consumption, it was thought essential to obtain data concerning the variation in normal oxygen consumption of the species and particular stock involved. The following tests were therefore made: 20 animals, ranging in weight from 1.9 to 3.2 grams, were selected and tested, individually, for four consecutive hours. It was found that movement of animals during hourly tests influenced normal oxygen consumption to a marked degree. Individuals, as a rule, if placed in flasks for two or three hours prior to testing, will settle to the bottom and remain motionless during subsequent hours. In cases where excessive movement did occur, however, the data obtained from such animals were discarded.

The results of this series are recorded in Table I. An examination of the data will show that no correlation exists between weight of individuals and the amount of oxygen consumed. Furthermore, the rises and falls in oxygen consumption from hour to hour do not follow a definite rhythm but fluctuate at random. The average percentage of hourly variation in oxygen consumption was arrived at

TABLE I

NORMAL VARIATION IN OXYGEN CONSUMPTION

Animal Number	Body Weight (Grams)	Oxygen in Cc. Consumed by Whole Animal during Consecutive Hours				Average Percentage Hourly Variation
		First	Second	Third	Fourth	
1	3.1	0.208	0.256	0.186	0.213	±21.9
2	2.8	0.472	0.498	0.391	0.316	15.2
3	2.7	0.537	0.547	0.567	0.507	5.4
4	2.5	0.305	0.348	0.275	0.228	17.4
5	1.9	0.394	0.303	0.288	0.396	21.7
6	2.9	0.427	0.391	0.273	0.273	12.9
7	2.2	0.178	0.231	0.221	0.261	17.9
8	3.0	0.269	0.297	0.206	0.314	31.1
9	2.0	0.304	0.299	0.199	0.227	16.4
10	2.1	0.563	0.556	0.607	0.465	11.2
11	2.1	0.489	0.524	0.481	0.612	10.8
12	2.5	0.493	0.519	0.443	0.493	10.3
13	3.2	0.251	0.263	0.258	0.265	2.6
14	2.8	0.538	0.475	0.396	0.321	16.4
15	1.9	0.739	0.673	0.562	0.518	11.0
16	2.2	0.551	0.468	0.462	0.404	6.3
17	2.3	0.326	0.395	0.273	0.197	26.6
18	2.7	0.557	0.647	0.562	0.470	15.2
19	3.1	0.219	0.238	0.168	0.258	30.7
20	2.0	0.285	0.383	0.323	0.351	19.5
Average		0.380	0.417	0.357	0.353	±16.5

by averaging the percentage variation between hours 1 and 2, 2 and 3, and 3 and 4. The final average obtained was 16.5 per cent, which represents the extent of variation in normal oxygen consumption for the species in question. From this it follows that any concentration of chloretone may be said to exert a true effect if the percentage variation between the amount of oxygen consumed in tap water and the amount consumed upon exposure to the chloretone solution is greater, on the average, than 16.5 per cent.

EFFECT OF VARIOUS CONCENTRATIONS OF CHLORETONE ON OXYGEN CONSUMPTION

The visible effects of exposing tadpoles to various concentrations of chloretone for an hour were first observed and recorded. These preliminary experiments were considered a necessary step in determining what concentrations of chloretone could be utilized for an hour, without lethal effects, for subsequent oxygen consumption tests. Solutions above 0.06 per cent were found to be lethal in all cases. The death-rate in 0.06 per cent solution was, on the average, 1 out of every 6 animals tested. For those that revived, the time required to respond to mechanical stimulation after an hour's exposure to this concentration was approximately three hours. Concentrations between 0.02 and 0.06 per cent chloretone produced anesthesia within one hour. Solutions of chloretone below 0.02 per cent were found to be incapable of producing anesthesia although the animals were kept in these solutions for several days.

The effects of non-anesthetizing and anesthetizing concentrations of chloretone on respiration were determined in the following manner: Animals were selected ranging in weight from 1.7 to 3.2 grams. From 10 to 12 of these individuals were then used for testing the effect of each concentration. The following strengths of chloretone solution were used: 0.00125, 0.0025, 0.00375, 0.005, 0.01, 0.02, 0.03, 0.04, and 0.06 per cent. In all, 103 animals were tested for four consecutive hours each. The first, third, and fourth hours were run in tap water while the second-hour test was made in the desired concentration of chloretone. The effect of the anesthetic was calculated in terms of percentage variation between the first, or normal hour, and the second, or chloretone hour. The complete data covering this series will be found in Table II.

The data reveal that solutions having the strengths of 0.00125, 0.0025, and 0.00375 per cent do not exert any marked effect on oxygen consumption since the average percentages obtained were typical of normal variation. The first effective concentration found was that of 0.005 per cent chloretone which depressed the oxygen consumption of 11 animals tested by 29.2 per cent, on the average. The threshold of effect, therefore, was thought to lie probably between 0.00375 per cent chloretone, which shows stimulations and depres-

sions within normal variation, and 0.005 per cent solution, which gives evidence of a true depression in all animals tested.

It was thought possible that the low concentrations of chloretone

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF CHLORETONE

Animal No.	Body Weight (Grams)	Cc. of O_2 Consumed During Successive Hours (Whole Animal)						Percentage Variation between Hours 1 and 2	Average Percentage Variation
		First Hour (Water)	(Second Hour) (Chloretone)			Third Hour (Water)	Fourth Hour (Water)		
			0.00125%	0.0025%	0.00375%				
1	2.4	0.399	0.515	.....	.....	0.421	0.411	29.2	
2	2.8	0.272	0.317	.....	.....	0.267	0.244	16.3	
3	3.1	0.596	0.684	.....	.....	0.729	0.502	14.7	
4	2.5	0.423	0.273	.....	.....	0.348	0.419	11.7	
5	3.0	0.614	0.481	.....	.....	0.499	0.477	21.5	
6	2.9	0.178	0.195	.....	.....	0.133	0.218	9.3	
7	1.9	0.436	0.365	.....	.....	0.381	0.421	13.8	
8	2.6	0.342	0.364	.....	.....	0.261	0.282	6.4	
9	2.6	0.250	0.268	.....	.....	0.234	0.263	6.9	
10	2.4	0.344	0.273	.....	.....	0.236	0.251	20.6	
11	2.6	0.449	0.502	.....	.....	0.505	0.481	11.6	
12	3.0	0.315	0.358	.....	.....	0.288	0.481	13.5	±14.6
1	2.8	0.368	.....	0.261	.....	0.324	0.400	29.0	
2	2.8	0.456	.....	0.418	.....	0.406	0.371	8.3	
3	3.1	0.438	.....	0.464	.....	0.449	0.403	5.8	
4	2.8	0.408	.....	0.376	.....	0.271	0.345	7.9	
5	1.7	0.363	.....	0.222	.....	0.141	0.167	38.8	
6	2.3	0.222	.....	0.219	.....	0.133	0.118	1.1	
7	2.7	0.427	.....	0.482	.....	0.378	0.320	12.8	
8	2.9	0.312	.....	0.282	.....	0.228	0.247	9.5	
9	2.1	0.423	.....	0.314	.....	0.346	0.339	23.5	
10	2.1	0.516	.....	0.368	.....	0.385	0.366	28.6	
11	2.5	0.308	.....	0.245	.....	0.236	0.239	20.5	16.9
1	2.5	0.573	.....	.....	0.566	0.617	0.475	1.1	
2	2.1	0.404	.....	.....	0.313	0.298	0.406	22.6	
3	2.6	0.557	.....	.....	0.567	0.587	0.527	1.8	
4	2.6	0.299	.....	.....	0.327	0.236	0.291	9.2	
5	3.2	0.303	.....	.....	0.267	0.256	0.308	11.6	
6	1.9	0.295	.....	.....	0.225	0.264	0.310	23.5	
7	2.6	0.531	.....	.....	0.440	0.468	0.538	17.2	
8	2.6	0.598	.....	.....	0.479	0.650	0.625	19.8	
9	2.3	0.290	.....	.....	0 274	0.229	0.289	5.6	
10	2.7	0.365	.....	.....	0.304	0.348	0.303	16.5	
11	2.9	0.408	.....	.....	0.467	0.367	0.307	14.4	
12	2.9	0.189	.....	.....	0.147	0.208	0.185	22.1	13.8

TABLE II—*Continued*

EFFECT OF VARIOUS CONCENTRATIONS OF CHLORETONE

Animal No.	Body Weight (Grams)	Cc. of O_2 Consumed during Successive Hours (Whole Animal)						Percentage Variation between Hours 1 and 2	Average Percentage Variation
		First Hour (Water)	Second Hour (Chloretone)			Third Hour (Water)	Fourth Hour (Water)		
			0.005%	0.01%	0.02%				
1	2.3	0.591	0.390	.....	.....	0.557	0.476	34.0	
2	3.2	0.654	0.384	.....	.....	0.561	0.472	41.2	
3	2.8	0.502	0.414	.....	.....	0.404	0.350	17.3	
4	2.5	0.565	0.454	.....	.....	0.547	0.557	19.6	
5	3.0	0.577	0.450	.....	.....	0.499	0.407	21.9	
6	2.1	0.487	0.421	.....	.....	0.471	0.317	13.5	
7	2.8	0.488	0.384	.....	.....	0.437	0.490	21.1	
8	2.9	0.339	0.206	.....	.....	0.267	0.269	39.2	
9	2.9	0.509	0.254	.....	.....	0.342	0.361	50.0	
10	2.3	0.499	0.292	.....	.....	0.409	0.462	41.4	
11	2.8	0.216	0.171	.....	.....	0.280	0.324	20.7	29.2
1	2.2	0.279	.....	0.176	.....	0.171	0.220	36.9	
2	2.6	0.357	.....	0.238	.....	0.173	0.427	33.0	
3	2.8	0.398	.....	0.210	.....	0.468	0.387	47.3	
4	2.8	0.459	.....	0.230	.....	0.169	0.282	49.6	
5	3.0	0.187	.....	0.146	.....	0.148	0.170	21.7	
6	3.0	0.277	.....	0.139	.....	0.163	0.260	49.8	
7	2.9	0.229	.....	0.128	.....	0.177	0.238	44.0	
8	2.8	0.343	.....	0.123	.....	0.127	0.175	64.0	
9	2.4	0.380	.....	0.160	.....	0.422	0.397	57.7	
10	2.4	0.346	.....	0.086	.....	0.166	0.299	75.0	
11	2.6	0.390	.....	0.070	.....	0.317	0.355	81.9	
12	3.0	0.428	.....	0.199	.....	0.296	0.444	53.5	51.2
1	2.5	0.370	.....	.....	0.082	0.260	0.383	77.5	
2	2.8	0.416	.....	.....	0.083	0.185	0.407	79.9	
3	3.1	0.410	.....	.....	0.081	0.267	0.411	80.1	
4	3.0	0.411	.....	.....	0.050	0.292	0.347	87.8	
5	2.9	0.368	.....	.....	0.117	0.118	0.415	68.1	
6	2.1	0.528	.....	.....	0.173	0.178	0.434	67.0	
7	2.8	0.467	.....	.....	0.165	0.201	0.413	64.6	
8	2.9	0.466	.....	.....	0.073	0.206	0.456	84.3	
9	2.9	0.318	.....	.....	0.096	0.203	0.410	69.7	
10	2.9	0.288	.....	.....	0.082	0.256	0.281	71.0	
11	1.9	0.370	.....	.....	0.084	0.229	0.294	77.3	
12	2.8	0.397	.....	.....	0.086	0.194	0.331	78.2	75.4

used might be exerting some effects on respiration, which were obscured by the magnitude of normal variation as at first calculated. A survey of normal oxygen consumption from hour to hour (Table

TABLE II—*Continued*

EFFECT OF VARIOUS CONCENTRATIONS OF CHLORETONE

Animal No.	Body Weight (Grams)	Cc. of O_2 Consumed during Successive Hours (Whole Animal)							Percentage Variation between Hours 1 and 2	Average Percentage Variation
		First Hour	Second Hour (Chloretone)				Third Hour	Fourth Hour		
		(Water)	0.02%	0.03%	0.04%	0.06%	(Water)	(Water)		
1	2.7	0.498	.....	0.039	.....	.....	0.038	0.037	92.0	
2	2.7	0.488	.....	0.117	.....	.....	0.096	0.317	75.9	
3	3.1	0.619	.....	0.075	.....	.....	0.034	0.327	87.8	
4	2.7	0.392	.....	0.087	.....	.....	0.061	0.150	77.8	
5	2.1	0.315	.....	0.028	.....	.....	0.051	0.450	91.0	
6	2.1	0.458	.....	0.065	.....	.....	0.032	0.214	85.0	
7	3.0	0.478	.....	0.052	.....	.....	0.088	0.301	89.0	
8	2.9	0.386	.....	0.068	.....	.....	0.068	0.327	82.2	
9	2.1	0.437	.....	0.064	.....	.....	0.022	0.171	85.2	
10	2.8	0.414	.....	0.115	.....	.....	0.030	0.132	76.6	
11	2.3	0.322	.....	0.089	.....	.....	0.020	0.129	72.7	83.4
1	2.6	0.264	.....	.....	0.042	.....	0.021	0.129	83.9	
2	2.8	0.275	.....	.....	0.074	.....	0.040	0.085	72.3	
3	3.1	0.366	.....	.....	0.064	.....	0.050	0.185	81.4	
4	2.9	0.600	.....	.....	0.045	.....	0.031	0.066	92.4	
5	2.0	0.417	.....	.....	0.026	.....	0.045	0.070	93.7	
6	2.1	0.529	.....	.....	0.083	.....	0.042	0.116	84.1	
7	2.8	0.476	.....	.....	0.040	.....	0.030	0.271	91.5	
8	2.5	0.326	.....	.....	0.061	.....	0.029	0.176	81.1	
9	3.0	0.203	.....	.....	0.034	.....	0.075	0.061	82.9	
10	2.5	0.316	.....	.....	0.053	.....	0.032	0.083	80.2	84.4
1	2.8	0.312	.....	.....	.....	0.025	0.024	0.031	91.8	
2	3.2	0.300	.....	.....	.....	0.022	0.021	0.048	92.1	
3	2.7	0.255	.....	.....	.....	0.011	0.013	0.053	95.7	
4	2.1	0.360	.....	.....	.....	0.026	0.047	0.058	92.6	
5	2.1	0.635	.....	.....	.....	0.050	0.026	0.057	92.1	
6	2.6	0.312	.....	.....	.....	0.045	0.029	0.034	85.4	
7	2.5	0.223	.....	.....	.....	0.027	0.027	0.045	87.6	
8	2.5	0.209	.....	.....	.....	0.057	0.048	0.054	72.6	
9	2.1	0.381	.....	.....	.....	0.023	0.020	0.014	93.8	
10	2.6	0.216	.....	.....	.....	0.025	0.029	0.022	88.4	
11	2.6	0.357	.....	.....	.....	0.069	0.036	0.036	80.6	
12	2.6	0.479	.....	.....	.....	0.060	0.020	0.022	87.3	88.6

I) shows that the variations which occur are negative and positive in nature; that the final percentage variation of 16.5 per cent represents only the average change in oxygen consumption, either up or down, for consecutive hours and which is not indicative of the extent of true positive and negative variations which occur from hour

to hour. A second method of calculation, based on the extent of true negative and positive variations, indicates that a concentration has a stimulating effect when the percentage of positive variations exceeds greatly the percentage of negative variations, or a depressing effect when the reverse is true, or normal variation when the positive and negative variations are such as to balance each other. On this basis, the results obtained in the normal series indicate average positive variations to the extent of 17.4 per cent and average negative variations amounting to 13.3 per cent, giving a final balanced average variation of +2.05 per cent. Theoretically, perfect normal variation would, of course, give a balanced variation of 0 per cent.

TABLE III

AVERAGE OF VARIATIONS

Solution	Positive (Per Cent)	Negative (Per Cent)	Positive+ Negative (Per Cent)
Normal series (tap water)	+17.4	−13.3	+ 2.05
0.00125 per cent chloretone	+13.4	−16.9	− 1.75
0.0025 per cent chloretone	+ 9.3	−18.6	− 4.65
0.00375 per cent chloretone	+ 8.5	−15.5	− 3.50
0.005 per cent chloretone	none	−29.0	−29.00

In a similar manner, the effects produced by chloretone concentrations of 0.00125, 0.0025, 0.00375, and 0.005 per cent were calculated. The results are given in Table III. The results show that the effect of these lower concentrations manifests itself by reducing the extent of positive variations. These data show also that with each rise in concentration, from 0.00125 to 0.00375 per cent, the extent of positive variations becomes smaller while (with one exception) there is an accompanying increase in the percentage of negative variations. It would seem, therefore, from this method of analysis, that both 0.0025 and 0.00375 per cent chloretone may have a slight depressing influence on oxygen consumption. The first concentration to produce a marked effect on oxygen consumption, however, is again shown to be 0.005 per cent chloretone, which effect manifests itself by obliterating all positive variations to produce an average depression of 29.0 per cent.

The results of the higher concentrations of chloretone (above 0.005 per cent) were characterized by a progressive depressing effect on oxygen consumption which became absolutely larger but proportionately smaller as the strength of the chloretone solution was increased (see Table II). The most pronounced average percentage depression (88.6 per cent) and the greatest individual percentage depression (95.7 per cent) were obtained when using 0.06 per cent chloretone. Intermediate concentrations between 0.005 and 0.06 per cent, or specifically, 0.01, 0.02, 0.03, and 0.04 per cent chloretone,

TABLE IV

COMPARISON OF MEAN DIFFERENCES AND THEIR STANDARD ERRORS

Means Compared	Difference between Means	Standard Error of the Difference	Difference / Standard Error of the Difference
Normal and 0.00125 per cent.....	1.9	2.49	0.77
Normal and 0.0025 per cent.....	0.4	3.83	0.10
Normal and 0.00375 per cent.....	2.7	2.78	0.97
Normal and 0.005 per cent.....	12.7	3.93	3.2
Normal and 0.01 per cent.....	34.7	5.02	6.9
Normal and 0.02 per cent.....	58.9	2.63	22.4
Normal and 0.03 per cent.....	66.9	2.54	26.3
Normal and 0.04 per cent.....	67.9	2.60	26.1
Normal and 0.06 per cent.....	72.1	2.45	29.4

depressed oxygen consumption, on the average, 51.2, 75.4, 83.4, and 84.4 per cent, respectively (see Table II).

It is of interest here to inquire whether or not application of the standard error serves to verify the conclusions reached in respect to the depressive effects recorded for the various chloretone concentrations used. In Table IV the mean average percentage variation, as determined for each concentration of chloretone and as recorded in Table II, is compared with the normal mean average percentage hourly variation of 16.5 as recorded in Table I.

If we apply the general rule that a difference between two means which is at least three times its standard error represents a significant difference, then we can conclude that chloretone concentrations from 0.005 to 0.06 per cent, inclusive, exercised a true depressive effect. Likewise, concentrations below 0.005 per cent cannot be said to have

a definite depressing effect on oxygen consumption. It will thus be seen that the conclusions reached through application of the standard error agree closely with those previously reached by simple comparison of the mean average percentage variations.

Data concerning the recovery from depressing concentrations of chloretone, as indicated by the oxygen consumption in tap water determined during the third- and fourth-hour tests, are shown in Table II. These data are summarized in Table V in terms of average percentage variation between the third and first hour in order to

TABLE V

SUMMARY OF NORMAL AND CHLORETONE SERIES

Percentage Chloretone	Number of Animals	Average percentage Variation between Hours		
		1 and 2	2 and 3	3 and 4
Tap water	20	12.6	17.3	14.6
0.00125 per cent	12	14.6	13.3	19.6
0.0025 per cent	11	16.9	17.9	11.8
0.00375 per cent	12	13.8	16.7	17.9
		1 and 2	1 and 3	1 and 4
0.005 per cent	11	29.2	15.6	20.0
0.01 per cent	12	51.2	39.2	15.0
0.02 per cent	12	75.4	44.7	11.1
0.03 per cent	11	83.2	85.5	54.8
0.04 per cent	10	84.4	87.9	65.1
0.06 per cent	12	88.6	90.6	88.2

show the extent of recovery by the end of the third hour. A similar comparison is made between the first- and fourth-hour averages to determine whether or not recovery is complete by the end of the fourth hour. It is evident from Table III that solutions of chloretone having strengths of 0.03, 0.04, and 0.06 per cent still exerted their effects during the third and fourth hours. These concentrations produced complete anesthesia for part of the second hour, all of the third hour, and, in the case of 0.06 per cent chloretone, for the entire fourth hour. Recovery, in the case of 0.01 and 0.02 per cent chloretone, was complete by the end of the fourth hour. In 0.005 per cent chloretone solution recovery was very rapid, being complete during the third hour. Thus, it is apparent, that in higher concentrations

(0.03, 0.04, 0.06 per cent) recovery is attained slowly, while in lower concentrations (0.005, 0.01, and 0.02 per cent) recovery is more rapid.

ACCLIMATION TO DEPRESSING EFFECT OF CHLORETONE

It was thought of interest to inquire whether or not the larvae might adjust themselves, in time, to the action of chloretone and thus exhibit a normal variation of oxygen consumption in the presence of a concentration which had previously been found to depress their respiration. Since 0.01 per cent chloretone had been shown to give a definite depression of 51.2 per cent, the attempt was made to acclimate larvae to this concentration. This concentration of chloretone was also particularly suitable in that it had previously been shown that larvae could remain alive in it practically indefinitely without becoming anesthetized. The following tests were consequently devised and carried out.

The individual oxygen consumption of 20 animals for one hour was first measured in tap water. Six individuals of this group were then used for acclimation purposes, while the remaining 14 were reserved as controls. The 14 animals set aside as controls were isolated for five days in 3-liter jars filled with water; the water being changed daily. At the end of the fifth day the oxygen consumption of 4 of these animals was measured, individually, for one hour in 0.01 per cent solution. The other 10 larvae were tested for oxygen consumption in tap water. The average percentage variation for the group tested in 0.01 per cent chloretone gave a depression of 55.0 per cent, while the second control group showed an average normal variation of 5.9 per cent.

The 6 test-animals were placed individually in three-liter rubber-stoppered jars containing 0.005 per cent chloretone for twenty-four hours. They were then transferred to 0.01 per cent chloretone solution in which they were maintained for four days. At the end of each day the jars were emptied and fresh 0.01 per cent solution added. Following the five-day acclimation period, these animals were then tested in a 0.01 per cent solution for one hour. The percentage variation between the initial tap-water oxygen consumption test and the chloretone oxygen consumption test of the fifth day was then calculated. The chloretone hour test in this case again showed a depres-

sion amounting to 65.8 per cent on the average. It is thus apparent that the test animals did not acclimate themselves to the effects of the 0.01 per cent solution, at least as far as oxygen consumption was concerned.

The inability of the animals to acclimate themselves to the depressing action of 0.01 per cent chloretone was thought to have been due, possibly, to the procedure employed. Two other methods were consequently devised, the first of which involved a longer exposure of the larvae to 0.01 per cent chloretone, while the second entailed a

TABLE VI

CHLORETONE CONCENTRATIONS USED IN ACCLIMATION TEST

Day	Percentage Chloretone	Day	Percentage Chloretone
1	0.001	6	0.006
2	0.002	7	0.007
3	0.003	8	0.008
4	0.004	9	0.009
5	0.005	10	0.01

gradual increasing in the strength of the chloretone solution during a similar, comparatively longer acclimation period. Separate control groups were also run with each series similar to the chloretone controls as used and described in connection with the earlier acclimation experiments.

In respect to the first of these two methods, 8 animals were first tested in tap water for one hour and then placed in 0.01 per cent chloretone for eleven days. Their oxygen consumption for one hour was then measured at the end of the eleventh day in 0.01 per cent chloretone. The average depression obtained here was 56.3 per cent. Four chloretone controls showed an average depression of 58.6 per cent. Here, again, the results give evidence of a lack of acclimation. The second method involved the following procedure: 8 animals, whose individual initial oxygen consumption for one hour in tap water was first determined, were placed in successively increasing strengths of chloretone solution during the next ten days, as shown in Table VI. On the eleventh day the animals were tested, individually, in 0.01 per cent chloretone for one hour. The average per-

centage variation between the amount of oxygen consumed during the initial tap-water test, prior to the acclimation period, and the amount consumed in 0.01 per cent chloretone on the eleventh day amounted to a depression of 63.1 per cent. Four chloretone control animals were depressed, on the average, 58.6 per cent. Again, the results obtained show no indication of an adjustment to the depressing action of 0.01 per cent chloretone. It may be concluded, therefore, that, with the methods employed, the larvae were unable to acclimate themselves as far as a depression in oxygen consumption was concerned.

DISCUSSION

The results of the present paper indicate that normal oxygen consumption of larvae of *Rana clamitans* is subject to considerable hour-to-hour fluctuation. To cite an extreme case, the average variation for four consecutive hours amounted to 31.1 per cent, while the fluctuation between two consecutive hours of this period was even greater. Although such a high degree of variation in oxygen consumption may seem somewhat abnormal, similar cases are on record involving as high and even greater changes in respiration. Thus Narins (1930), using the same species of frog tadpole, points out a variation of 38 per cent in one case, while Helff (1928) records even larger fluctuations to be true for normal adult crayfish. Although this fluctuation in oxygen consumption of aquatic animals appears to be so prevalent among certain groups of animals as to be almost axiomatic, it must still be admitted that the fluctuations vary, in general, around a definite mean. This would certainly indicate that some form of regulatory mechanism must be operating although, as far as maintaining a constant intake of oxygen is concerned, it would appear to be quite imperfect. It should be said, however, that although the gross intake of oxygen into the blood stream certainly varies greatly over stated time intervals, it does not necessarily mean that this same fluctuation is true of the actual oxygen consumption of the various body tissues. The exact nature of respiratory regulation, as far as oxygen consumption is concerned, is therefore still practically unknown for aquatic amphibians and presents a problem of considerable interest although difficult of solution.

Within a certain range of concentration, chloretone solutions quite

clearly depress oxygen consumption, markedly. In general, it may be said that as the strength of the chloretone solution applied becomes progressively greater, the depression in oxygen consumption becomes absolutely larger although proportionately smaller. It is also interesting to note that the effect of chloretone in the higher concentrations, especially, is not transitory but, in fact, tends to persist and even to increase in amount following an initial exposure of the animal to the solution. Thus, the results are quite clear in that oxygen consumption is still depressed during the next hour, and in some cases the next two hours, following the chloretone hour and during which respiration is being carried on in tap water. It would seem as though either the tissues of the larvae absorb and retain the chloretone for some time and so supply an internal depressive action on oxygen consumption or else the initial depressive effect on the respiratory mechanism governing oxygen intake is so pronounced that considerable time is necessary for the mechanism to adjust itself even when the larvae are placed in a normal water environment.

Regardless of the mechanism involved, the fact that chloretone has been shown to actually depress oxygen consumption would seem to lend emphasis to Harrison's (1904) and Mathews and Detwiler's (1926) explanations for the general retardation in embryonic development and the reflex pattern which they describe as true for frog and *Amblystoma* embryos following immersion in chloretone solutions. If, as they suggest, the retardation is the result of a lowering of general metabolism brought about through action of the chloretone, we should expect a decrease in oxygen consumption, which the results of the present paper show to be the case. Harrison's observations to the effect that, in general, the degree of retardation is proportional to the strength of the chloretone solution applied, are likewise more understandable in the light of the present results regarding the relationship between depth of oxygen depression and concentration of chloretone. It may be of interest here, also, to compare the relative depressing influence of chloretone with that of chloroform. Narins (1930) has shown in the case of chloroform that 0.01 and 0.125 per cent solutions depress oxygen consumption of larvae of *Rana clamitans* 30.6 and 67.5 per cent, respectively. The present results, using chloretone, show approximately similar depressions of

29.2 and 88.6 per cent when using solutions of only 0.005 and 0.06 per cent, respectively. It is apparent, therefore, that chloretone possesses an unusually strong depressive influence on the oxygen consumption of the larvae in question.

The threshold of depressing effect with chloretone undoubtedly is to be found in as weak a solution as 0.005 per cent. In this connection we may regard concentrations of 0.00125, 0.0025, and 0.00375 per cent chloretone as exerting either no effect or a slight depressive effect, depending on the method of analysis employed, as previously pointed out. The writer is of the opinion that the second analytic method is logical and hence that the true threshold of depressive effect probably lies nearer 0.0025 per cent than 0.005 per cent chloretone. As has been pointed out before, however, the degree of normal variation in oxygen consumption characteristic of the species makes an accurate determination of the true threshold quite difficult, if possible at all.

The acclimation experiments as performed in the present work apparently did not result in an acclimation of the larvae to the depressive effects of chloretone in the concentration employed. It should be noted, furthermore, that in some cases there were suggestions of an additive depressive effect when the larvae were left for several days in the chloretone solutions. However, it is just possible that the larvae were unable to acclimate themselves due to the abrupt change in strength of chloretone concentrations from day to day during a comparatively short period for acclimation. It is possible, although the writer thinks rather improbable, that a very gradual and slow increase in the strength of the chloretone solution (as could be obtained by the addition of a 0.01 per cent solution drop by drop to a large quantity of water over, say, a month's time) might result in an acclimation to the depressive action on oxygen consumption.

SUMMARY AND CONCLUSIONS

1. The normal oxygen consumption of 20 tadpoles (*Rana clamitans*) was determined individually for four consecutive hours. The extent of hourly variation was found to be, on the average, 16.5 per cent. This normal variation was utilized in evaluating the effect of various concentrations of chloretone on oxygen consumption.

2. Animals were tested for four consecutive hours, the second-hour test being made in the desired concentration of chloretone. From 10 to 12 animals were used to determine the effect of each concentration. Chloretone solutions of 0.005, 0.01, 0.02, 0.03, 0.04, and 0.06 per cent were found to depress oxygen consumption markedly, the average percentage depression being 29.2, 51.2, 75.4, 83.4, 84.4, and 88.6 per cent, respectively. The threshold of effect was recorded when using 0.005 per cent chloretone. Recovery was attained slowly, subsequent to the use of higher concentrations and more rapidly following treatment with lower concentrations.

3. The influence that certain low concentrations of chloretone (below 0.005 per cent) might have on oxygen consumption was determined. Solutions of 0.00125, 0.0025, and 0.00375 per cent were found to produce variations in oxygen consumption of 14.6, 16.9, and 13.8 per cent, respectively. A second method of analysis, involving the balancing of negative and positive variations, indicated that these concentrations produce average variations in oxygen consumption of -1.7, -4.6, and -3.5 per cent, respectively, while the variation for the normal series calculated by the same method amounted to $+2.0$ per cent. While this comparison might serve to indicate a slight depressive effect of 0.0025 and 0.00375 per cent chloretone on oxygen consumption, it should be borne in mind that statistical treatment of the results (see Table IV) do not substantiate such a conclusion.

4. Several experiments were devised in an attempt to induce acclimation of larvae to the depressing influence of 0.01 per cent chloretone on oxygen consumption. These, in general, involved the constant immersion of larvae in a chloretone solution whose strength was increased over several days' time until a concentration of 0.01 per cent was attained. Subsequent oxygen consumption tests in 0.01 per cent chloretone were largely negative in that no acclimation to the depressive effect of 0.01 per cent chloretone on oxygen consumption was obtained.

5. The results are discussed in general, and the relationship of oxygen depression is correlated with previously determined effects of chloretone on embryological development in Amphibia.

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THE VIABILITY OF TAPEWORMS IN ARTIFICIAL MEDIA

(Two figures)

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THE experimental precision with which investigations into the physiology of free-living Platyelmia are conducted has not been found generally applicable to the endoparasitic members of the phylum. Even in the most favorable media the life of such animals is short, and they are extremely susceptible to tissue disintegration induced by the constituents of the medium and by the microflora of the medium. Relatively little attention has been paid to the possibility of overcoming the experimental difficulties thus provided, and in consequence the physiology of endoparasitic flatworms is very inadequately understood, and published information concerning them is predominantly morphological in nature.

The problem which confronts the student of tapeworm cultivation is that of maintaining a larval or an adult worm outside its host, for a period of time approximating to its normal longevity *in vivo*, in a condition in which it remains, so far as can be judged, normal in shape and movement, and in which it continues to grow and to mature. The problem thus demands: (1) the establishment and maintenance of an aseptic or an oligoseptic medium, whether a molecular solution or a colloidal sol or gel, which can serve as a carrier for the carbohydrate and nitrogenous requirements of the animal; (2) the elimination of micro-organisms from the surface of the animal during its transference from host to medium; and (3) the provision of the appropriate food requirements of the animal.

In experiments of this nature the animal selected should preferably be of a size convenient to handle, should be available in quantity, should be relatively hardy outside its host, and should show a measurable periodicity of movement. Such requirements are satisfied more readily by a larval than by an adult tapeworm, and the subject selected for the experiments described below was a tetra-

rhynchid larva, identical probably with *Nybelinia surmenicola* Okada. Larvae of this genus have been repeatedly described and figured by European authors, and reference may be made to Dollfus (1930) for a full discussion of the morphology and synonymity of the larva and to Pintner (1930) for a description of the adult worm. The writer (Wardle, 1932) has previously recorded and described this larva from *Ophiodon elongatus*, a hexagrammid fish common in British Columbia coastal waters. Twenty per cent of these fishes, of four years old or more, are infected with the larva, which occurs wandering freely over the stomach mucosa, or imbedded within it, in numbers varying from 20 to 100 per infected fish. It was thus possible to obtain and utilize several thousand larvae.

The occurrence of a tapeworm, whether larval or adult, in the stomach of an animal is unusual, except in an encysted condition, as the high hydrion concentration of gastric juice discourages their establishment. In wide-mouthed, wide-throated, bottom-feeding fishes, such as *Ophiodon*, however, sea water may enter the stomach very readily and the hydrion concentration of the stomach contents may be relatively low. A series of pH determinations of the stomach mucus of *Ophiodon*, made with a B.D.H. Capillator, gave for fasting fishes pH values of 3.8–4.0, and for fishes with partially digested stomach contents, values of 7.0–7.5. The pH values of the sea water in the cages in which the fishes were retained until killed for commercial sale varied between 7.5 and 8.5.

There are certain objections to the removal of a cestode directly from its host habitat into the medium whose influence is being studied. The animal is usually coated with contaminated mucus which may obscure any injuries inflicted during the removal, and its movements tend to be irregular. It is preferable to keep the newly secured cestode for some time in a medium which is antagonistic to mucous and to microfloral contamination, and which can serve as a solvent for antiseptic or saline or nutrient substances. The initial medium made use of in the following experiments was double-strength, sugar-free, Ringer-Locke's solution (NaCl 1.8 per cent, KCl 0.8 per cent, $CaCl_2$ 0.04 per cent, $NaHCO_3$ 0.04 per cent), buffered to a pH value of 7.5 Nybelinid larvae will live at least six days in this medium and during that time will show little evidence of dis-

integration, distortion, or microfloral contamination. The animals were therefore transferred from the host-fish, usually within two or three hours after the fish was killed, directly into this medium and kept there for one hour. Then, those individuals which appeared clean, vigorous, uninjured, and whose rhythmic contractions approximated 20 per minute, were selected for the experiments. This medium served also as a solvent for all the antiseptic substances tested, and for many of the nutrient substances tested. Decomposition of the carbonate content by heat sterilization did not seem to make the medium less favorable, but carbonate-free medium was distinctly inferior. In the presentation of the data the initial medium will be referred to simply as double Locke.

The media used in the experiments comprised (*a*) saline media, (*b*) antiseptic media, and (*c*) nutrient media. The influence of each medium upon survival time, upon rate of movement, and upon tissue disintegration, was observed. The aseptic experiments were carried out in sterilized 100 mm. Petri dishes. The oligoseptic experiments were carried out in Syracuse dishes. In each case only two larvae per dish were used. When more larvae were used they showed a tendency to crowd together and to anchor themselves to one another by their proboscides. Each experiment was repeated three times, the majority being repeated six or eight times. The dishes were kept in a subdued light at laboratory temperatures.

Obvious sources of experimental error are suggested by the low number of larvae used per experiment, by the absence of temperature control, and by the absence of hydrion control. In favorable media, such as double Locke, the maximum survival time among 12 individuals in a Petri dish was not perceptibly greater than that of two individuals in a Syracuse dish, conditions of temperature, light, and concentration of medium being equal; but in unfavorable media there was a slight difference in favor of the crowded individuals. It is, of course, well established that certain animals will survive much longer in an uncongenial environment as groups than as solitary individuals, and the possibility of such group-protection occurring also among cestodes is a point worthy of investigation.

The temperature of the environment of *Ophiodon*, which is a relatively deep water fish, during June and July, 1932, ranged from 8° to

10° centigrade (bottom temperatures, Porlier and Active passes, Straits of Georgia, B.C.), and the body temperatures of the fish can be assumed to be not more than 2° higher than those of the surrounding sea water. The laboratory temperatures under which the experiments were conducted ranged from 18–24° C. It is certain, therefore, that the survival times recorded for the cestodes are lower, and the rates of movement higher, than would have been recorded if the media had been kept at temperatures approximating to those of the host habitat. The absence of hydrion control should not be a serious source of error, since the majority of the media employed had pH values within the range, 3.8–7.5, which occurs in the *Ophiodon* stomach, but the possibility cannot be overlooked that the rate of penetrations of the ions of the medium, into the worm, may vary with the hydrion concentration.

However, no claim is put forward that the data recorded represent absolute values of survival time and rate of movement in the media tested, but they are presented here as indicating the relative favorableness of certain media toward nybelinid larvae, and as offering some indication of the guiding principles to be used in further experiments in the cultivation of cestodes *in vitro*.

The investigations were carried out in the laboratories of the Pacific Biological Station, Nanaimo, British Columbia, during June, July, and August, 1932, and the author is greatly indebted to the Biological Board of Canada and to the Station Director, Dr. W. A. Clemens, for the facilities so generously placed at his disposal. The author is indebted also to Dr. H. B. Ward, of the University of Illinois, for many fruitful suggestions.

SALINE MEDIA

Several observations scattered through the scanty literature of cestode physiology refer to the influence of aqueous media upon the viability of tapeworms.

Among early references are those of Frisch (1734), that the plerocercoid stages of *Schistocephalus solidus* can live more than two days in river water; of Fabricius (1780) that "Taenia erythrini" (=*Proteocephalus percae* Müller) from *Perca norvegica* can live several weeks in sea water; of Abildgaard (1793) that "Taenia gasterostei" (=ple-

rocercoid of *Schistocephalus solidus* Müller) can live eight days in fresh water; of Dujardin (1837) that isolated proglottids of "Taenia cucumerina" (=*Dipylidium caninum* Linn.), "Taenia serrata" (=*Taenia pisiformis* Bloch), "Taenia filicollis" (=*Proteocephalus percae* Müller) and "Taenia fringillarum" (=*Hymenolepis fringillarum* (Rud.), will live several days in a saturated atmosphere. Among later observations, Le Bas (1924) has recorded the viability of plerocercoids of *Diphyllobothrium latum* Linn. for seven days in Ringer-Locke's solution. Cook and Sharman (1930), investigating the influence of acids and bases upon the respiratory rate of *Moniezia trigonophora* Stiles and Hassall, and using carbonate-free Ringer's solution

TABLE I

HOURS OF SURVIVAL TIME OF LARVAL *Diphyllobothrium latum* IN SALINE MEDIA

MEDIUM	MOLAR CONCENTRATION				
	0.2	0.1	0.05	0.01	0.001
Sodium chloride	30.0	40.0	70.0	4.0	2.0
Potassium chloride	3.5	3.5	18.0	4.5	1.0
Calcium chloride	60.0	28.0	12.0	3.0	2.0
Sodium bicarbonate	8.0	12.0	15.0	1.25	0.05
Sodium carbonate	5.3	7.3	12.0	15.5	5.5

as a solvent for the acids and bases tested, report the following hours of survival time at 37° C.: M/10,000 NaOH, 286; carbonate-free Ringer, 200; M/10,000 HCl, 175; M/1000 NaOH, 120; M/1000 HCl, 90; distilled water, 48; M/100 NaOH, 44; M/100 HCl, 36; M/10 NaOH, 0; M/10 HCl, 0; tap water, 0.

Wardle (1932*b*) investigating the influence of various saline media upon the viability of the plerocercoid stage of *Diphyllobothrium latum* at 38° C. records the hours of survival time as given in Table I.

To these data may be added the following unpublished values of survival time for the plerocercoid stage of *Triaenophorus tricuspidatus* (Bloch) and for the adult stage of *Bothriocephalus scorpii* (Müller) in saline media, at temperatures fluctuating around 20° C.: *Triaenophorus tricuspidatus:* NaCl M/5, 48+:M/10, 48+:M/20, 48+:M/100, <1:M/1000 <1:$CaCl_2$ M/5, 20+:M/10, 20+:

M/20, 1–2:M/100, <1:M/1000 <1:KCl M/5, 5–6:M/10, 1–2: M/20, <1:M/100 <1:M/1000, < 1:distilled water < 1. *Bothriocephalus scorpii:* Ringer-Locke's solution, 406–432:Ringer-Locke less bicarbonate, 192–216: double Ringer-Locke, 96:quadruple Ringer-Locke, <2:sea water, 36–48:NaCl M/10, 36–48:KCl M/10, 8–10:$CaCl_2$ M/10, <2:saline citrate, 12–16, Mines saline, 0:distilled water, <2:tap water <2.

Scattered and incomplete though the recorded observations may be, they suggest, at any rate, that balanced saline solutions such as Ringer-Locke and sea water, and to a lesser extent, concentrations of sodium chloride between M/20 and M/5, are tolerated by a tapeworm better than are low molecular concentrations of their component salts, or than is fresh water.

In the present experiments the saline media employed comprised:

(1) A series of physiological salines consisting of *sugar-free Ringer-Locke*:*sugar-free Ringer-Locke less bi-carbonate*:*saline phosphate* (NaCl 0.64 per cent, KCl 0.033 per cent, CaCl 0.016 per cent, Na_2HPO_4 0.017 per cent, NaH_2PO_4 0.001 per cent): *saline citrate* (NaCl 0.7 per cent, $2Na_3C_6H_5O_7 \cdot 11H_2O$ 1 per cent: *Mines saline; seawater;* and for comparison, *tapwater* and *distilled water*.

(2) A series of single salines, namely, *sodium chloride*, *potassium chloride*, *calcium chloride*, *magnesium chloride*, *sodium bicarbonate*, made up (*a*) in concentrations of M/1–M/100 and (*b*) in the percentage concentrations in which they appear in double Locke.

The media were unchanged throughout the experiments.

The minimum and maximum values of survival time, expressed in hours, of *Nybelinia* in these media are given in Table II.

The conclusions that can be drawn from the data in Table II are that balanced saline solutions are more favorable to the viability of nybelinid larvae than are their component salts. Among the balanced salines, single and double Ringer-Locke are about equally favorable; saline citrate, sea water, and quadruple Ringer-Locke, form a second group about equally favorable but less so than the first group; bicarbonate-free Ringer-Locke, saline phosphate, and half-strength Ringer-Locke form a third group, equally favorable, but less so than the first two groups; tap water, distilled water, and Mine's saline are unfavorable.

Among the single salines, NaCl in concentrations of M/5–M/10 is about equal to the second group in favorableness; the other salines

TABLE II

HOURS OF SURVIVAL TIME OF LARVAL *Nybelinia surmenicola* IN SALINE MEDIA

(The concentrations in Series 1 are the standard formulas; in Series 2, they are molar concentrations; in Series 3, they are as in double Ringer-Locke solution.)

MEDIA	CONCENTRATIONS						
	As Given	Molar	Molar/2	Molar/3	Molar/5	Molar/10	Molar/100
Series 1:							
Locke×½	60–72						
Locke×1	96–340						
Locke×2	144–288						
Locke×4	112–120						
Locke less $NaHCO_3$	60–65						
Sea-water	48–144						
Sal. citrate	48–120						
Sal. phosphate	48–96						
Mines saline	1–2						
Tap-water	1–2						
Dist. water	0.5–1.0						
Series 2:							
NaCl		0.5 –1.0	14–24	24–30	48–96	72–144	2–5
KCl		0.5 –1.0	2–5	7–8	7–8	3–8	2–5
$CaCl_2$		0.16	0.16–0.25	0.5–1.0	12–18	0.6–1.0	2–4
$MgCl_2$		0.00	0.5 –1.0	1–2	2–4	7–8	2–4
$NaHCO_3$		0.00	3–4	3–4	3–4	48–72	2–3
Series 3:							
NaCl 1.8 per cent	48–72						
KCl 0.08 per cent	2–3						
$CaCl_2$ 0.04 per cent	1–2						
$NaHCO_3$ 0.04 per cent	0.25						

are unfavorable; the order of toxicity appears to be $CaCl_2 > MgCl_2 > KCl > NaHCO_3 > NaCl$.

ANTISEPTIC MEDIA

Few observations appear to have been published concerning the influence of antiseptic substances upon cestode viability. Coutelen

(1927) has recorded the death of larval *Echinococcus granulosus* within three minutes when exposed to mercuric chloride (1:1000) and to formol (1:200). Clapham (1932) has recorded the death of *Hymenolepis murina* within periods of 1, 2, 3, 4, and 5 minutes when exposed to hexyl resorcinol of concentrations of 1:1000, 1:5000, 1:10,000, 1:15,000 and 1:20,000 respectively, in an aqueous solution of 0.5 per cent methyl alcohol.

No attention appears to have been paid to the possibility of opposing the attack of micro-organisms upon cestodes, when *in vitro*, by the addition of antiseptic substances to the medium.

TABLE III

HOURS OF SURVIVAL TIME OF LARVAL *Nybelinia surmenicola* IN ANTISEPTIC MEDIA

(The antiseptic substances were dissolved in double Locke solution, the minimum concentrations being determined by bactericidal effect, the maximum concentrations, up to 10 per cent, being determined by solubility.)

Substance	Percentage concentrations					
	0.0001	0.001	0.01	0.1	1.0	10.0
Flavine	Non-bactericidal		68–72	68–72	3–4	0.0
Resorcinol	60–70	144–240	75–80	75–80	0.25–0.5	0.0
Formol	168–200	192–265	200–212	74–92	0.25–0.5	0.0
Phenol	*non-bact.*	120–130	120–130	2–3	0.25–0.5	0.0
Thymol	*Non-bactericidal*		180–200	0.16–0.25	*Insoluble*	
Chlorazene	109–144	120–130	15–64	0.25–0.30	0.0	0.0
Iodine	212–224	90–100	60–70	*Insoluble*		
Pot. permanganate	70–80	192–216	0.2–0.3	0.2 –0.3	0.25–0.50	0.0
Mercuric chloride	70–80	6–8	1–2	0.0	0.0	0.0

In the present experiments, the following antiseptic substances were tested, namely: *chlorazene, flavine, formol, iodine, mercuric chloride, phenol, potassium permanganate, resorcinol, thymol.* Each substance was dissolved in double Locke in concentrations ranging from one part per million to one hundred thousand parts per million of solvent, the minimum concentration being in each case the minimum bactericidal concentration, and the maximum concentration, up to 100,000, being limited by the solubility of the substance in the medium. The media were changed at intervals of 24 hours. The survival time values expressed in hours are shown in Table III.

A point of interest in the table of data is that the weakest concen-

tration of potassium permanganate and resorcinol, a concentration of only 0.0001 per cent, is apparently more lethal to the cestode than is the next higher concentration. It must be noted, however, that the lowest concentration represents in each case a molecular concentration below M/1000, and that of all the antiseptic concentrations tested, the lowest concentrations of these two substances were the only antiseptic media to induce muscular paralysis and complete relaxation of the animal. That is to say, potassium permanganate and resorcinol, in concentrations below M/1000, behave like similar concentrations of electrolytes of low lethality, such as NaCl, KCl, $CaCl_2$, HCl (cf. Wardle, 1932*b*).

The order of lethality of the range of antiseptics tested, towards *Nybelinia*, is mercuric chloride > potassium permanganate > iodine > chlorazene > thymol phenol > formol > resorcinol > flavine.

In a second and third series of experiments larvae were transferred to double Locke after immersion for one hour and for two hours respectively in those antiseptic concentrations which they could tolerate for those periods of time. The survival time values need not be given here since in the great majority of cases the values obtained were no greater, and in some cases were actually lower, than those of larvae left in the antiseptic. In the case of those individuals which were transferred from resorcinol (0.1 per cent) and thymol (.01 per cent), however, the survival time values approximated those of the control larvae in double Locke.

To test the inhibitive powers of antiseptic concentrations upon flora of the cestode surface, larvae which had been kept in double Locke for one hour were transferred to sterile media consisting of 3 per cent gelatine solution plus the appropriate antiseptic concentration. The depth of liquefaction of each medium was measured at daily intervals. At the end of seven days all the media showed liquefaction except those containing flavine (1 per cent) and thymol (0.1 per cent), but the extent of liquefaction was slight in those media containing flavine (0.1 per cent), chlorazene (0.1 per cent), and phenol (0.1 per cent). A second series of media was inoculated with stomach mucus from a freshly killed *Ophiodon*. At the end of six weeks all the media showed microfloral contamination except those containing formol (0.2 per cent), thymol (0.01 per cent and 0.1 per cent), phenol

(0.1 per cent), resorcinol (1 per cent), but only slight contamination was shown by the media which contained flavine (0.4 per cent).

The conclusions that can be drawn from the data presented above are that the survival time of larval *Nybelinia* in double Locke is adversely affected by all the antiseptic concentrations tested. Taking the average value of survival time in unchanged double Locke as 192 hours, the least departure from this value was induced by the presence of formol (0.01 per cent or less), iodine (0.0001 per cent), potassium permanganate (0.001 per cent), resorcinol (0.001 per cent), and thymol (0.01 per cent). None of these concentrations, however, was able to inhibit microfloral contamination, with the exception of thymol (0.01 per cent), which was definitely inhibitive.

Thymol, resorcinol, and formol merit further investigation.

NUTRIENT MEDIA

There are several published references to the viability of tapeworms in nutrient media.

Knoch (1863) observed that the scolices of bothriocephalid cestodes from fishes will live eight days in an aqueous solution of egg albumin. Pintner (1881) recorded that many cestodes from marine fishes will live five to six days in sea water plus a trace of egg albumin. Zschokke (1888) kept cestodes from selachian fishes alive for 24 hours in sea water plus host gut-mucus. Lönnberg (1892), using *Triaenophorus tricuspidatus* from the gut of *Esox lucius*, succeeded in keeping individuals alive for three to four weeks in a dilute, slightly acid, pepsin-peptone solution of sodium chloride, the medium being changed frequently to counteract microfloral contamination; when cane sugar was added to the medium, the animals only lived fourteen days; in a peptone solution alone they lived only four days; the experiments were conducted at 10° C. in semi-darkness; at 18–20° C., in a lighted room, the experiments were unsuccessful. Tower (1900) kept *Moniezia expansa* alive for five days in a medium composed of 10 per cent albumin, 5 per cent beef extract, 2 per cent glucose, in tap water.

In none of the experiments recorded above, however, was there any indication of growth or development on the part of the worms.

Dévé (1926) has recorded the viability of larval *Echinococcus granu-*

losus in aseptic hydatid fluid plus fresh, unheated horse serum and he obtained an increase in volume of some of the scolices. Coutelen (1927, 1929), using a similar technique, has followed the development of the scolices of the same larval cestode speciés for thirty-one days in a medium of hydatid fluid plus ascitic serum, until they attained a volume 25–35 times that of the original volume; individual coenurids of *Multiceps serialis* remained alive for twenty days and doubled in volume when kept aseptically in sodium chloride solution plus fresh horse serum, renewed daily, at 37° C.

It must be emphasized that no accurate information is available concerning the food requirements of a cestode or the method by which it satisfies those food requirements. It is generally assumed that the food consists of such cell-permeable products of the host digestive powers as aminoacids, glucose, and glycerine; that the cestode cannot utilize fats, disaccharides, polysaccharides, proteins, and hydrolyzed proteins; and that the foodstuffs enter the cestode by diffusion through the body surface. In the absence of experimental verification of these assumptions, however, any attempts to feed cestodes *in vitro* cannot be otherwise than empirical.

Some support to these assumptions, however, has been provided by an accumulation of evidence concerning the physico-chemical nature of the *milieu intérieur* of larval taeniids (*Echinococcus granulosus*, *Taenia hydatigena*, *Multiceps serialis*) brought forward by Flössner (1923–25), Van der Hoeden (1925), Mazocco (1923), Schilling (1904), Wernicke and Savino (1923), Schopfer (1932), to mention only recent contributors to the question. The most important contributions are those of Schopfer (1932) to whom reference may be made for a critical résumé of the results of the contributors mentioned.

There is a general agreement among these investigators that the fluid within the inner membrane of the cysticercid or the coenurid type of larval cestode is a transudate from the blood plasma of the host, and is poorer in proteins and richer in chlorides than is the host blood plasma. The poverty in proteins is regarded by some authors as evidence that the cestode assimilates proteins, but the most recent work of Schopfer suggests, on the other hand, that the metabolism of the cestode larva is not sufficiently intense to use up proteins so rap-

idly, and that the protein poverty of the internal milieu is due to the low permeability of the inner membrane to proteins of the host plasma. The fundamental nutritive substance appears to be, above all, glucose.

The extent to which cestodes can absorb substances which are in colloidal dispersion, such as fats, is uncertain. That adult cestodes have a high fatty-acid content is well known, and the earlier workers, such as Schiefferdecker (1874), postulated the direct passage of fat globules through cuticular pores in the cestode surface. Brand (1931) has shown, by chemical analysis, that in *Moniezia expansa* the protein content (3.95 per cent) is relatively low, and the glycogen (2.69 per cent) and fat (3.35 per cent) content is relatively high, as compared with other animals; *Moniezia*, and possibly other cestodes, is unique among animals in having a glycogen-fat content in excess of the protein content. The fat content consists of phosphatides (15 per cent), stearin (7 per cent), stearic acid (8 per cent), oleic acid (51 per cent), higher oxy-fatty acids (14 per cent), and glycerol (4 per cent), and Brand regards the fat content as being of the nature of an accumulated excretory product, a by-product of glycogen degradation. Under anaerobic conditions, at 39° C., *Moniezia* in Ringers solution loses 0.25 per cent of its glycogen content in six hours but loses none of its fat content. The amounts of carbon dioxide (0.11 per cent), succinic acid (0.01 per cent), lactic acid (0.05 per cent), higher fatty acids (0.05 per cent) excreted into the medium, nearly balance the glycogen loss and so support the view that the fatty acid excretion is a result of glycogen degradation. Brand claims also that sugar-fed cestodes show an increase in internal fat content.

In the present experiments, the following nutrient media were employed:

(1) *Guyénots medium, Locke-bouillon, serum saline.*

(2) *Double Locke* plus 0.25 per cent of one of the following: *glucose, dextrin, gelatine, glycerine, egg albumin, soluble starch, peptone.*

The Guyénots medium was a modification of that used by Guyénot (1917) for *Drosophila*, the tributyrin and yeast of the original formula being omitted. The Locke-bouillon comprised four volumes of double Locke, one volume of fish bouillon, 0.25 per cent glucose, 0.25 per cent of glycerine, and was buffered to a pH of 7.5.

The serum-saline consisted of saline citrate solution plus 0.5 per cent of Loeffler's dehydrated blood serum.

These three media were employed (*a*) as fluids, under aseptic conditions; (*b*) as fluids, under oligoseptic conditions, the medium being changed daily; (*c*) as gels, by the addition to them of 3 per cent of gelatine, under aseptic conditions; (*d*) as fluids upon a base of double Locke-agar gel, fluid and base being changed daily; and (*e*) as fluids

TABLE IV

HOURS OF SURVIVAL TIME OF LARVAL *Nybelinia surmenicola* IN NUTRIENT MEDIA

(In Series 1, the media were used (*a*) as aseptic fluids; (*b*) as oligoseptic fluids; (*c*) as aseptic gels, with 3 per cent of gelatine; (*d*) as oligoseptic fluids on a base of double Locke-agar gel; and (*e*) as oligoseptic fluids on a base of host stomach mucosa. Series 2 were all used as oligoseptic fluids.)

	a	*b*	*c*	*d*	*e*
Series 1:					
Guyénot's medium	130–150	130–150	96–120	120–144	120–144
Locke bouillon	180–200	130–150	120–168	120–144	168–192
Serum-saline	108–120	108–120	144–192	120–144	120–144
Double Locke (control)	432–456	250–270	.....	120–144	140–164
Double Locke gel (control)	.....	.....	120–168	.....	.....
Agar gel (control)	.....	.....	.....	168–192	.....
Series 2:					
Double Locke+glucose, 0.25 per cent		360–408			
Double Locke+dextrin, 0.25 per cent		48–96			
Double Locke+gelatine, 0.25 per cent		48–72			
Double Locke+glycerine, 0.25 per cent		120–216			
Double Locke+egg albumin, 0.25 per cent		144–240			
Double Locke+starch, 0.25 per cent		60–70			
Double Locke+peptone, 0.25 per cent		60–70			
Double Locke (control)		250–270			

upon a base of *Ophiodon* stomach mucosa, the fluid only being changed daily.

The media in the second group were used under oligoseptic conditions only. Double Locke was the control medium; the larvae were kept for one hour in double Locke, and for 15 minutes in flavine (1 per cent), before transference to the medium. Each set of experiments was repeated three times.

The survival time values, expressed in hours are given in Table IV.

The majority of the experiments were brought to a conclusion by the death of the larvae through microfloral attack, the preliminary immersion in flavine not being effective in inhibiting this, and there is somewhat of a uniformity in the survival values obtained, since the hour of death of a larva suffering from fungal or bacterial disintegration is difficult to estimate.

The survival time value of larvae in sterilized, unchanged double Locke is noteworthy, since it is almost double the value when this medium is changed daily, and is in opposition to the suggestion that death in an unchanged medium might be caused by the accumulation of fatty acids in the medium.

In no case was any increase in volume of the cestode observed even in the media—serum saline gel, Locke bouillon, and Locke-glucose—in which the highest values of survival time were obtained. The greatest success, from the standpoint of retention of normal appearance and movement by the animals, rather than actual longevity, was obtained with nutrient gels under aseptic conditions. Upon an agar-gel base, for example, the animals looked as normal, as smooth, as plump, regular in movement, as larvae living *in situ*, and if the successful sterilization of such larvae, prior to transference to the medium, could be attained, such nutrient gels would seem to offer the best possibilities for cestode culture.

DISCUSSION

Owing to the relative arrangement of the longitudinal and circular musculature, the movements of an adult tapeworm, or of a plerocercoid larva, consist of alternating waves of contraction and expansion. These contractions and expansions are not of uniform intensity throughout the strobila but appear to be at a maximum in that region of the animal where growth is most active, that is to say, in the region of maximum metabolic activity. In a tetrarhynchoid cestode, this is the so-called Keimzone or "neck"; in dibothriocephaloid cestodes, in which new proglottids arise by transverse division of the proglottids of the abothrial half of the primary strobila, the region of maximum activity lies approximately at the junction of the first and second thirds of the strobila. The strobila of a cestode is thus alternately shortening and lengthening toward, or away from, this

region of maximum metabolism, and as the region lies nearer to the bothrial extremity than to the abothrial extremity, each expansion gives the animal an impetus in a bothriad direction.

Such movements are particularly vigorous in a short-bodied muscular cestode such as a nybelinid or a diphyllobothriid larva, when it is placed in a non-lethal medium of low viscosity. In the case of *Nybelinia*, for example, in double Locke, the waves of movement commence in a region midway between the posterior bothrial margin and the edge of the caudal invagination, and are so vigorous that at each expansion the animal doubles its *in situ* length. The friction between worm and substratum, necessary for forward progression, is provided by the temporary adherence of one or other bothrium to the bottom of the dish, and by the push of the abothrial extremity against the bottom of the dish, and the animal can move forward with a slight rotating movement. Such forward progression is not possible in a long strobiliform cestode, owing to the relative inertia of the abothrial half of the strobila, but in muscular form such as *Eubothrium oncorhynchi*, *Bothriocephalus scorpii*, *Bothriocephalus cuspidatus*, the expansions and contractions of the scolex and of the anterior proglottids are most pronounced, and the scolex may expand to twice or three times its *in situ* length; in other forms, however, such as *Triaenophorus tricuspidatus*, the movements are comparatively sluggish.

When the tapeworm is *in situ* in the host gut, however, movement is damped by the viscosity of the gut contents, by the pressure of the scolex against the gut mucosa, by the peristalsis of the gut wall, and, in a long cestode, by the inertia of the abothrial half of the strobila. The cestode is therefore usually in a condition of natural tonus, of partial contraction, and the rhythm of movement is slow and irregular. The larval *Nybelinia*, *in situ*, expands at a mean rate of 4 expansions per minute, as compared with a rate of 24–30 expansions per minute in double Locke. This natural tonus serves to maintain the pressure between scolex and mucosa, and, in a segmented strobila, to maintain the integrity of the proglottid junctions, against the backward drag and disruptive tendency of gut peristalsis.

If, through starvation, or through the pressure of the maturing genitalia upon the musculature, or through toxic elements in the

host gut, or other causes, this tonicity cannot be maintained, the cestode may be expelled *in toto*, or as weakened fragments of strobila, or even as isolated proglottids, from its habitat. Such expulsion of immature individuals of *Eubothrium*, *Bothriocephalus*, *Diphyllobothrium* from the host gut is not uncommon; the expulsion of aged, sexually exhausted cestodes from the host habitat is probably the normal termination of the cestode life-cycle; the phenomenon of *pseudapolysis*, the expulsion of exhausted strips of strobila, is a common occurrence with *Diphyllobothrium latum* in human or canine hosts; the passage of mature or of immature proglottids from the host is a normal phenomenon of the taenioid and tetrarhynchoid life-cycles. The larvae of *Phyllobothrium salmonis*, common as an accidental parasite of the gut of the Pacific salmon (*Oncorhynchus*) is apparently unable to counteract the peristalsis of its habitat and so passes down to the host rectum where it accumulates and may be expelled from the salmon in such numbers, when the fish is artificially stripped of ova, as to clog the sieves. On the other hand, if the opposition of the host to cestode movement is weak, as in a diseased or moribund host, or in an abnormal host, the impetus of the cestode may throw it into coils and folds, a condition familiar to the collector of cestode material, or may move it cephalad along the host gut, probably an uncommon occurrence, or, as in the case of tetrarhynchoid larvae occurring as accidental parasites in the stomach of teleostean fishes, may help to drive it through the mucosa into the underlying tissues.

When a cestode is removed from its host and placed in an artificial medium, its behavior, in terms of movement and distortion, may be one of four types, a statement which is based upon observation of the rate of movement of *Nybelinia* larvae timed with a stop-watch, supported by observations upon *Gilquinia squali*—a tetrarhynchoid common in the Pacific dogfish (*Squalus suckii*)—*Eubothrium oncorhynchi*, *Bothriocephalus scorpii*, *Bothriocephalus cuspidatus*, and the plerocercoid stages of *Diphyllobothrium latum* and *Triaenophorus tricuspidatus*. No observations have been made by the writer on taenioid, phyllobothrioid, or proteocephaloid cestodes.

The term *normal*, as given below, is meant to refer to the rate of movement of the cestode when *in situ*.

I. The rate of movement may fall to a value slightly subnormal, and remain fluctuating around that value—the cestode being in natural tonus—until terminated by the death of the animal from starvation or from microfloral attack. No distortion nor premortem disintegration occurs. Such behavior has been observed only in the case of *Nybelinia* larvae when upon solid media such as agar-gel and serum-saline gel.

II. The rate of movement may rise to a high supernormal value and then decline slowly over a period of days or even weeks to a subnormal value, whereupon the animal goes into tetanic contraction and undergoes fractional disintegration, death and disintegration proceeding from one or both extremities and from the outer surface inward (Fig. 1).

Such behavior is shown in NaCl, KCl, $MgCl_2$, $NaHCO_3$, HCl, when the concentration of the electrolyte lies between M/100 and M/2. The activity period may be a matter of minutes, hours, days, even weeks; the contraction phase may be brief, as in $CaCl_2$, or may be prolonged, as in HCl.

The relative favorableness of a saline medium toward a cestode should be estimated therefore rather by the length of the activity phase than by the whole survival period, since the animal cannot recuperate from the contraction phase.

In contracted plerocercoids of *Diphyllobothrium latum*, disintegration commences at the abothrial extremity and proceeds forward, and the scolex may be contracting and expanding rhythmically when the tissues behind it are in a state of disintegration. In the plercercoid of *Nybelinia*, on the contrary, disintegration commences at the bothrial extremity and proceeds backward, and the caudal invagination may be undergoing rhythmic inversion and eversion when the scolex is a swollen, disintegrating mass. In adult *Bothriocephalus scorpii* individual disintegration proceeds rapidly from the abothrial extremity forward, and less rapidly from the bothrial extremity backward; the movements of the anterior region of the strobila in opposition to the relative inertia of the posterior region of the strobila, may throw the strobila into a tight spiral, and the slower movements of the strobilar margins, as compared with

the mid-dorsal and mid-ventral regions of the strobila, may produce "frilling," the margins being partially expanded and pleated, while the middle of the region is contracted.

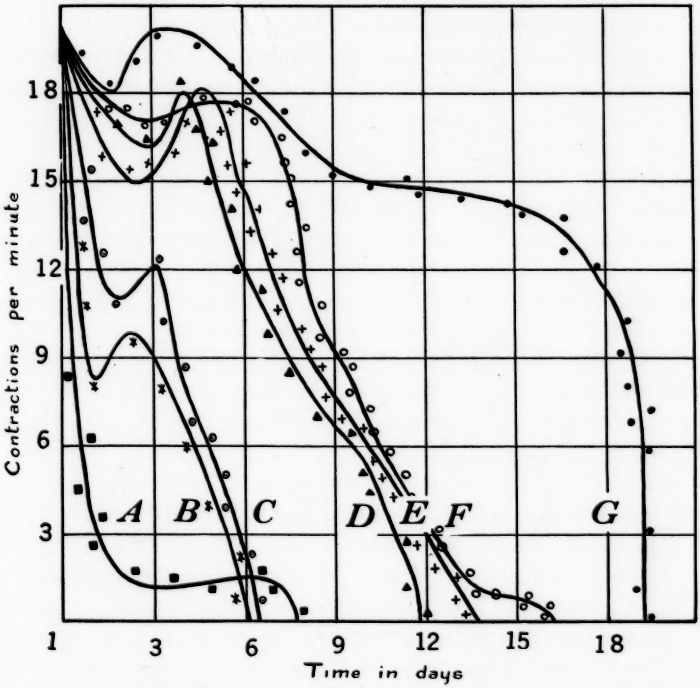

FIG. 1.—Curve illustrating the rates of movement of larval *Nybelinia surmenicola* in favorable media and showing first a fall in the initial rate, followed by a recovery, followed by a gradual decline to zero. *A*, serum saline gel; *B*, seawater; *C*, NaCl M/10; *D*, Locke solution×2, unsterilized; *E*, Locke solution×1, unsterilized; *F*, Locke solution×2+glucose, unsterilized; *G*, Locke solution×2, sterilized.

III. The animal may pass quickly into a condition of tetanic contraction. Such is the case in NaCl, KCl, $MgCl_2$, HCl, in molar and supermolar concentrations, and in the case of *Nybelinia*, in all the antiseptic media tested, except resorcinol, in concentrations equal to or greater than: chlorazene (0.01 per cent), flavine (1 per cent), formol (0.4 per cent), phenol (0.1 per cent),

mercuric chloride (0.0001 per cent), iodine (0.01 per cent), potassium permanganate (0.1 per cent). Death in this phase is

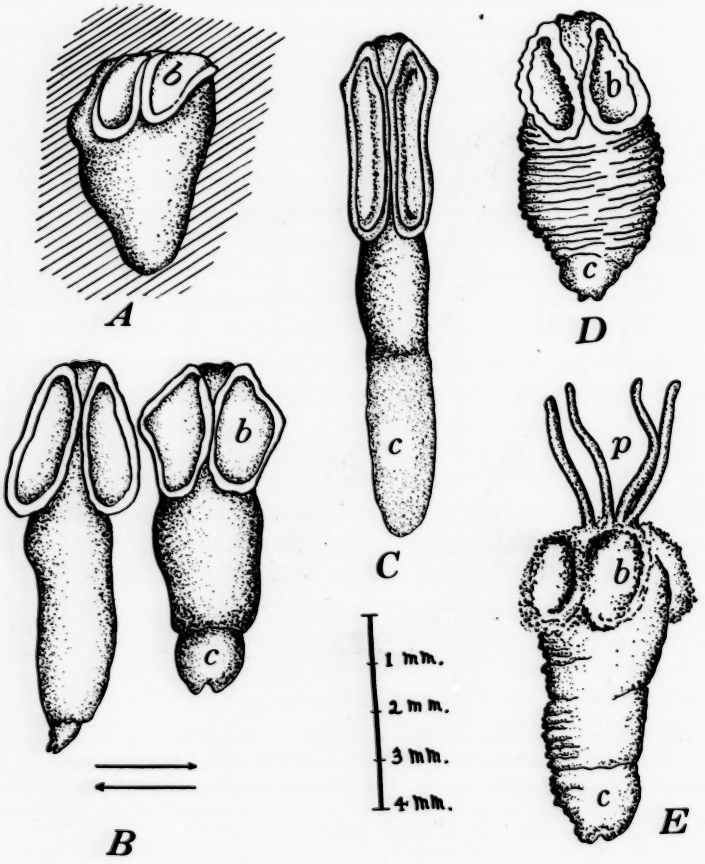

FIG. 2.—The morphological appearance of larval *Nybelinia surmenicola* in various media. *A*, appearance *in situ; B*, in favorable salines; *C*, extreme expansion in resorcinol; *D*, in tetanic contraction; *E*, in endosmosis and disintegration; *b*, bothria; *c*, caudal appendage; *p*, proboscides.

followed by endosmotic bloating but further dissolution may be inhibited in an antiseptic medium.

IV. The animal passes quickly into a condition of extreme relaxation, and then proceeds to disintegrate with great rapidity. Such rapid relaxation is induced by water, by NaCl, KCl, $MgCl_2$, in concentrations below M/100, by calcium compounds, by sodium and potassium hydroxides, and by resorcinol (Fig. 2).

The variations in behavior shown by cestoda in different artificial media are such as to suggest that the cestode surface is permeable to, and its tissues susceptible to, the lethality of the ions OH′, H′, Ca″, K′, MG″, Na′ in the order given. The effect of these ions penetrating into the *milieu intérieur* of the cestode is first to inhibit the region of maximum metabolic activity, H′, K′, Mg″, Na′ inducing tonic muscular contraction, OH′ and Ca″ inducing muscular relaxation. There occurs, therefore, as these ions penetrate, a tonic contraction of the region of maximum activity of worms in salines containing sodium, potassium, magnesium, and a paralysis or relaxation of this region of worms in pure water, in low concentrations of salines, or in calcium salines. The extremities of the worm, furthest away from the inhibited region, will continue to show rhythmic activity, but eventually death and disintegration will commence in the tissues farthest away from the inhibited region and proceed gradually toward it. Such a hypothesis explains all types of cestode behavior in aqueous media, and is in accordance with the findings of Child (1924, 1928) and his collaborators in the case of free-living flatworms, where the effect of sublethal media is first to inhibit the regions of greatest metabolic activity and then gradually to inhibit the regions of minimum metabolic activity; the regions of maximum metabolism recuperate somewhat, however, but the regions of minimum activity do not recuperate so that death and disintegration commence in those tissues farthest from the region of maximum activity and proceed toward it.

The question arises as to whether the order of apparent lethality of ions toward the cestodes observed is really an order of relative toxicity, or is merely an order of relative penetrability. Is Ca″ really more toxic to cestodes than K′ or Na′ or does it appear so because it penetrates into the *milieu intérieur* more rapidly? No satisfactory answer can as yet be given to this question.

There is general agreement among workers in this field that the

first effects of electrolytes in a medium upon an organism are to alter the normal permeability of the body surface membrane and, in a poikilosmotic organism, to induce rapid endosmotic or exosmotic distortion, according to the relative concentrations of the electrolyte on either side of the membrane; and, second, to penetrate into the protoplasm and change it from sol to gel. The evidence concerning the subject is conflicting. In homoiosmotic organisms the distortion is less rapid, an osmotic balance being maintained for a time by energy induced by an increased oxygen intake (Schlieper *b*, 1929, Beadle, 1931).

Lillie (1909, 1910) found the surface permeability of *Arenicola* larvae to be increased by Na′, and to a lesser extent, by K′, but to be decreased by Ca″. Spek (1921) found the protozoan *Actinosphaerium eichhorni* permeable to K′, less so to Na′, and still less so to Ca″. Chambers and Reznikoff (1926) from a comparison of amoebae immersed in salt solutions of given concentrations with amoebae into which salt solutions of the same concentrations had been injected, arrived at the conclusion that NaCl and KCl are more toxic to the exterior of the cell than to the interior, whereas the reverse holds good in the case of $CaCl_2$ and $MgCl_2$. Mast (1931), however, has argued that the two sets of conditions are not comparable, since in the case of immersed amoebae a much larger area of protoplasm is exposed to the action of the salt than is the case when a minute quantity of the salt solution is injected into the animal. Now NaCl and KCl enter cells freely so that if an amoeba be immersed in a given concentration of either of these salts, the internal concentration will soon become much greater than if the salts are injected in minute quantity, and immersion will therefore be more injurious than inoculation to the animal. On the other hand, $CaCl_2$ and $MgCl_2$ enter cells very slowly so that their internal concentration in an immersed amoeba will increase more slowly than if the salts be injected. Therefore an inoculated animal will be more rapidly injured than an immersed animal. Mast finds that the relative toxicities of Na′, Mg″, Ca″, K′, NH_4′ to *Amoeba proteus* differ only slightly and that the relative toxicity of the different salts depends upon the concentration, Na′ being more toxic than Ca″ in concentrations of N/20 to N/500 and less toxic in a concentration of N/1000. Koidsumi (1931),

on the other hand, investigating the influence of salts upon the larvae of *Chironomus viridicollis* finds the toxicities of isotonic solutions of salts to vary according to the nature of their dissociated ions, the relative order of toxicity among kathions being $Hg'' > H', > Cu' > Cu'' > Co' > Li' > Mn'' > Fe'' > Zn'' > Ca'' > Ba'' > NH_4' > Mg'' > K' > Sr' > Na'$, and among anions $OH' > CO_3'' > NO_3'' > Cr_2O_4'' > SCN' > HPO_4' > CrO_4'' > I' > B' > Cl' > SO_4''$.

Hess (1930), with *Planaria dorotocephala*, finds KCl more toxic than NaCl, the oxygen consumption in concentrations of 0.04 molar, 0.02 molar, and 0.01 molar KCl rising rapidly on exposure of the animal and continuing to rise until death ensues, whereas, in NaCl, the respiratory rate, after a temporary increase due to increased muscular activity, remains around the normal level. To $CaCl_2$ in concentrations of 0.02–0.002 molar, the planarian is extremely tolerant. Hess suggests that the difference in toxicity between K and Na is due to a differential penetration, the surface membrane of the animal behaving like a collodion membrane and being almost impermeable to K' as compared with H', and almost impermeable to Na' as compared with K'.

A similar tolerance of calcium by free-living flatworms has been demonstrated also by Pantin (1931) for *Gunda ulvae*, which in the presence of calcium salts can control its permeability to water and to electrolytes far more effectively than it can in calcium-free water.

In view of such tolerance of calcium by free-living Platyelmia, the apparent susceptibility of *Nybelinia surmenicola* larva and of *Bothriocephalus scorpii* to calcium salts is worthy of further investigation, since it may throw a light upon the function of the calcareous bodies in cestode tissues and upon the extreme susceptibility of some cestodes to calcification *in situ*. It may be noted that with the plerocercoids of *Diphyllobothrium latum* and *Triaenophorus tricuspidatus*, intermuscular parasites of fresh-water fishes, no undue susceptibility to calcium salts was observed, and in the course of an examination of several thousand of these larvae the author has observed no case of calcification or partial calcification of such plerocercoids by the host tissues.

CONCLUSIONS

1. Balanced salines are more favorable to *Nybelinia surmenicola* larvae than are their component salts, the order of favorability being double Locke > single Locke > sea water > quadruple Locke > saline citrate > saline phosphate. Mines saline, tap water, and distilled water are rapidly lethal. The maximum survival time value was 456 hours in sterilized double Locke. Among the saline components in concentrations of M/5–M/10 the order of favorability was NaCl > $NaHCO_3$ > KCl > $MgCl_2$ > $CaCl_2$, the maximum value of survival time being 144 hours in NaCl M/10.

2. The survival time in double Locke is adversely affected by the presence of each of a range of antiseptic substances tested in concentrations of one part to one hundred thousand parts per million of solvent. The least diversion from the value in the control medium (double Locke) was induced by the presence of formol (.01 per cent), iodine (.0001 per cent), potassium permanganate (.001 per cent), resorcinol (.001 per cent), and thymol (.01 per cent), but none of these concentrations, except thymol (.01 per cent), was able completely to inhibit microfloral attack.

3. Of a range of nutrient media tested, the highest values of survival time were obtained in serum-saline-gel (192 hours), sterilized Locke-bouillon (200 hours), and Locke-glucose (408 hours); but the most promising media appear to be nutrient gels used under aseptic conditions. In no medium was any indication of larval growth observed.

4. The variations in behavior shown by *Nybelinia* and other Cestoda in artificial media suggest that the cestode surface is permeable to, and its tissues susceptible to the lethality of, the ions OH′, H′, Ca″, K′, MG″, Na′ in the order given, and that the effect of these ions is to inhibit the region of maximum metabolic activity, H′, K′, Mg″, Na′, inducing tonic muscular contraction, OH′ and Ca″ inducing muscular relaxation. There occurs, therefore, as these ions penetrate, a tonic contraction of the region of maximum activity of worms in salines containing sodium, potassium, magnesium, and a paralysis or relaxation of this region in worms in pure water, in low concentrations of salts, and in calcium salts. The extremities of the

worm farthest from the inhibited region continue to show rhythmic activity, but eventually death and fractional disintegration commence in the tissues farthest from the inhibited region and proceed gradually toward it. The behavior of Cestoda in saline media is in accordance with the findings of Child (1928) and his collaborators in the case of free-living flatworms.

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PHYSIOLOGICAL STUDIES CONCERNING THE REGULATION OF HEARTBEAT IN FRESH-WATER MUSSELS[1]

(Six figures)

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SEVERAL observers (Philippson, Hannevart, Thieren, 1910; Ellis, Merrick, and Ellis, 1931) have shown that definite changes in the salt content of the blood of fresh-water mussels can be induced quite rapidly by merely altering the salt content of the water in which the mussel is living. In view of the well-established importance of the salt balance in the maintenance of cardiac activity in most animals and the fact that the salt content, pH, and other physical and chemical properties of the water in which fresh-water mussels regularly live are subject to frequent changes, it would seem that the heart of the fresh-water mussel is either not sensitive to the changes in the salt content of the blood induced by the changes in environment, or that the heart is able to adjust itself readily to variations in the salt content over a rather wide range. The present experiments consider this problem, together with the reactions of the fresh-water mussel heart to various intrinsic and extrinsic factors.

MATERIAL

The hearts of 1,012 fresh-water mussels representing 45 species from 27 localities on the Mississippi River and various tributaries were used. The experimental studies were made aboard the U.S. Bureau of Fisheries floating laboratory, Quarterboat 348, during the 1931 summer cruise, and in the physiological laboratories of the University of Missouri, to which live mussels were shipped from various points. (See Table I for details of species studied.)

[1] The writer wishes to express his obligation to the United States Bureau of Fisheries for the material used in these studies, and his indebtedness to Dr. M. M. Ellis for the helpful advice given during the course of these investigations.

TABLE I

SPECIES OF MUSSELS STUDIED

Scientific Name	Common Name	Number of Individuals
Actinonaias carinata (Barnes)	River mucket	9
Amblema costata Rafinesque	Three ridge	2
Amblema peruviana (Lamarck)	Blue point	45
Anodonta corpulenta Cooper	Stout floater	1
Anodonta grandis Say	Floater	1
Arcidens confragosa (Say)	Rock pocketbook	1
Cyclonaias granifera (Lea)	Purple warty-back	9
Cyprogenia irrorata (Lea)	Fan shell	3
Elliptio crassidens (Lamarck)	Elephant's ear	13
Elliptio dilatatus Rafinesque	Lady finger	1
Fusconaia ebena (Lea)	Niggerhead	43
Lampsilis anodontoides (Lea)	Yellow sandshell	42
Lampsilis fallaciosa Simpson	Slough sandshell	134
Lampsilis higginsii (Lea)	Higgin's eye	1
Lampsilis ovata (Say)	Grandma	2
Lampsilis ventricosa (Barnes)	Pocketbook	1
Lasmigonia complanata (Barnes)	White heelsplitter	2
Leptodea fragilis (Rafinesque)	Fragile papershell	21
Ligumia recta (Lamarck)	Black sandshell	4
Megalonaias gigantea (Barnes)	Washboard	32
Obliquaria reflexa Rafinesque	Three-horned warty-back	17
Obovaria olivaria (Rafinesque)	Hickory nut	9
Plagiola lineolata (Rafinesque)	Butterfly	9
Plethobasus cyphyus (Rafinesque)	Bullhead	4
Pleurobema coccineum (Conrad)	Pink niggerhead	7
Pleurobema solida (Lea)	Small niggerhead	40
Properta alata (Say)	Pink heelsplitter	3
Properta capax (Green)	Fat pocketbook	3
Properta laevissima (Lea)	Fragile heelsplitter	2
Quadrula cooperiana (Lea)	Southern pimpleback	3
Quadrula cordata (Rafinesque)	Ohio River pig toe	99
Quadrula cylindrica (Say)	Rabbit foot	5
Quadrula fragosa (Conrad)	Hickory nut shell	2
Quadrula metanevra (Rafinesque)	Monkey face	132
Quadrula nodulata (Rafinesque)	Warty-back	26
Quadrula plena (Lea)	Pig toe	1
Quadrula pustulosa (Lea)	Pimple-back	151
Quadrula quadrula Rafinesque	Maple leaf	73
Quadrula subrotunda (Lea)	Long solid	1
Quadrula trapezoides (Lea)	Bank climber	2
Symphynota compressa Lea	Heel-splitter	2
Tritogonia verrucosa (Rafinesque)	Pistol grip	23
Truncilla donaciformis (Lea)	Fawn's foot	28
Truncilla truncata Rafinesque	Deer toe	2
Utterbackia imbecillus (Say)	Paper pond shell	1

METHODS

The fresh-water mussel heart is located on the dorsal side of the animal just beneath the hinge uniting the two shells, slightly more

posterior than anterior and just anterior to the posterior adductor muscle (see Fig. 1). When the pericardium is opened the heart may be seen as a rather delicate structure, with numerous intricately interlaced bundles of fibers making up the walls of the auricles and ventricle. Anatomical studies (Simpson, 1884; Willem et Minne, 1898; Motley, 1933) have shown that the heart of the fresh-water mussel is quite simple in structure, consisting of one ventricle in the mid-dorsal line, and two very thin-walled, incompletely separated auricles, a right and a left. The auricles are transparent, more or less pyramidal in shape, and are connected through a common auriculo-ventricular valve with the ventricle.

The mussel was opened by cutting the two adductor muscles at their insertions on the inside of one valve of the shell, and after removing the loose valve the heart was exposed by making a median longitudinal slit in the pericardium. The cut edges of the pericardium were retracted and at the same time elevated by tiny hooks of steel wire attached to silk threads. In this way the general contour of the pericardial cavity was maintained without loss of pericardial fluid. Kymograph records were obtained by attaching a light heart lever to the ventricle by silk thread and a tiny steel wire hook.

When external perfusion was desired, the perfusate was introduced into this cavity through a fine glass cannula, so arranged that the flow of fluid did not mechanically disturb the heart as it floated in the pericardial cavity. The heart was, therefore, completely surrounded at all times by either the pericardial fluid or the perfusion fluid. Internal perfusion was accomplished by inserting a fine hypodermic needle through the ventricular wall close to its base near the intestine, and allowing two or three drops of the perfusion fluid, which flowed into the needle from properly adjusted Mariotte bottles, to enter the ventricular cavity each minute.

NORMAL HEART ACTIVITY

As a background for the studies on the regulation of the heart large series of normals were obtained for all of the species used and correlations with age, species-size, species-activity, and various extrinsic factors made. Two of these correlations are given here in some detail. The others need only be summarized briefly.

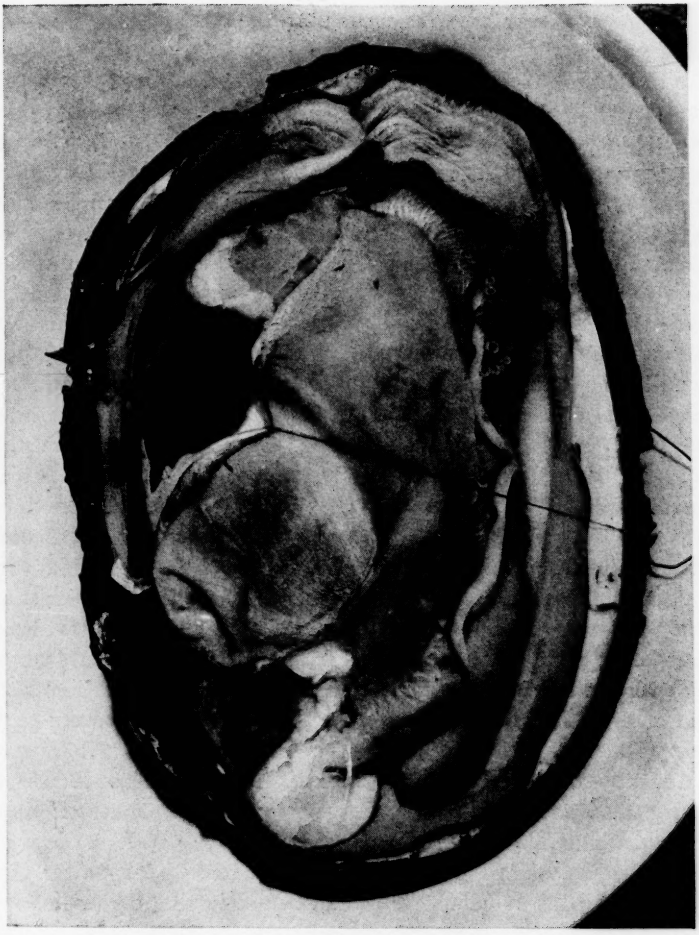

FIG. 1.—*Megalonaias gigantea*. Photograph of a living eight-year-old individual, left valve removed exposing the heart *in situ*. The pericardium has been cut and retracted showing the left auricle and the ventricle. Heart in diastole at time photograph was taken.

THE EFFECT OF OPENING THE SHELL ON HEART ACTIVITY

In general the exposed heart passed through four periods, from the time the shell was opened until the heart finally died, as regards type and rate of beat. In the first period, which lasted for only a few minutes immediately after the shell was opened, the beats were regular and even. During the second period, which varied with the species from two to twenty-four hours, the heartbeat became irregular and erratic. The third period was characterized by the return of a regular uniform heartbeat, very similar in rate, rhythm, and amplitude to the beat of the first period. The length of the third period varied from two to ninety-three hours. The fourth period began with the onset of fatigue, and was usually indicated by a reduction in the amplitude of the beat. As fatigue progressed a further reduction of amplitude occurred, accompanied by a gradual slowing of the rate, and finally a loss of the regular rhythm. These conditions persisted until the heart pulsations completely ceased. The hearts of *Megalonaias gigantea* were conspicuous in having a long second period, in practically every case twenty to twenty-four hours elapsing before the heartbeat of individuals of this species became regular in rhythm, rate, and amplitude, while on the other hand the hearts of *Lampsilis anodontoides* usually became regular within two to three hours after exposure. The exposed fresh-water mussel heart, varying with the species, continued to beat from twenty-four hours to four or five days after removal of the shell, if the animals were kept in a moist chamber (see Figs. 2 and 3).

As far as could be determined the irregular pulsations of the heart during the second period were the result of a partial contraction of the heart musculature. It would seem, therefore, that the validity of observations made on the heart immediately after the mussel had been subjected to the shock of cutting the adductor-muscles and opening the pericardium, might be questioned, as disturbances of the foot muscle and of the viscera might set up reactions which in turn might be reflected onto the heart mechanism and thus obscure the correct value. However, by checking the initial heart rate on opening the shell with the heart rate after a regular rhythm had been established in the third period, it was demonstrated that the heart count taken immediately after the heart was exposed was

valid, and that the opening of the shell produced little if any immediate shock on the cardiac mechanism. In view of these findings the irregularities of heartbeat during the second period were ascribed to loss of blood and to the visceral adjustments which always follow the opening of the shell.

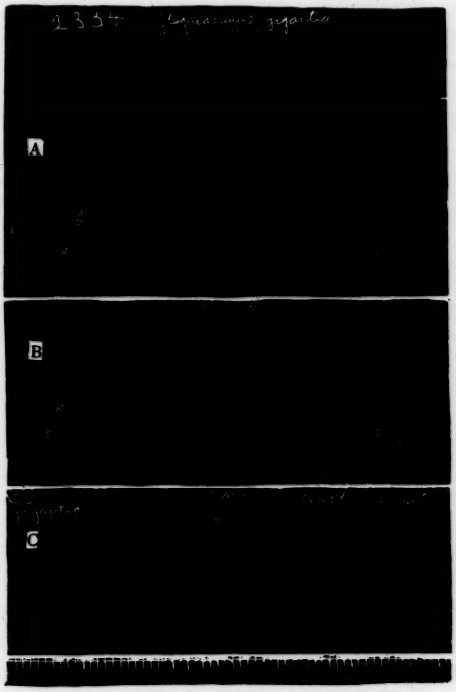

FIG. 2.—*Megalonaias gigantea*. Age seven years, kymograph records from ventricle, heart *in situ*. Temperature 25° C. Time interval five seconds. *A*, Cardiac activity, immediately after opening the pericardium. *B*, Thirty minutes later. *C*, Twenty-six hours after opening pericardium.

In connection with these periods of varying heart activity in the fresh-water mussel the findings of two previous observers may be mentioned. Baker (1897) studying the heartbeat of certain *Pelecypoda* through the pericardium described the heart action as remarkably regular in some individuals and quite erratic in others.

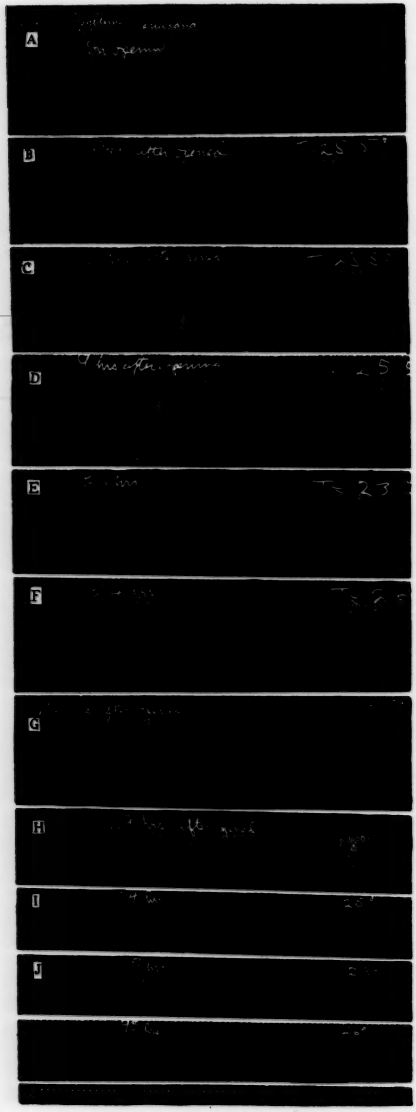

FIG. 3.—*Amblema peruviana*. Age six years, kymograph records showing the several periods of heartbeat as modified by length of exposure and temperature. Heart *in situ* in pericardial fluid, without perfusion. Time interval five seconds, all records taken from ventricle.

Simpson (1884) working with *Anodonta* stated that the ventricle does not contract and expand regularly in every portion at once, but that the wave of contraction spreads over the heart in a manner similar to peristalsis, the ventricle assuming various shapes from nearly spherical to elongate-ovoid. In the present studies this type of contraction was often observed shortly after opening the shell.

THE EFFECT OF TEMPERATURE ON HEART ACTIVITY

The action of temperature on the heart of the fresh-water mussel was followed in two ways: (1) by holding the mussels in properly aerated water of known temperature for several hours before opening the animals, and (2) by changing the temperature of the fluid in the pericardial chamber directly or by means of perfusion.

In general the response of the heart to changes in temperature as measured by heart rate seemed to be a straight-line function. The heart rate, within physiological limits, varied directly with the temperature instead of following the logarithmic type of curve, which some authors believe to be the curve of correlation of heart rate and temperature, and which would be expected were the van't Hoff rule applied.

The optimal temperature for the mussel's heart in most instances was about 30° C. As the temperature approached 40° C. there was usually a definite decrease in amplitude of contraction, the beats becoming irregular and somewhat periodic with intervals of little or no cardiac activity. At low temperatures, 15° C. to 4° C., the declining heart rate was accompanied by a decrease in amplitude and often by an irregular rhythm. Below 5° C. the heartbeat was usually suppressed, but in some cases beats occurred at irregular intervals at temperatures as low as 2° C. Contraction and relaxation were about equally affected. The hearts regularly recovered from low temperature exposures without any apparent loss of function, the process evidently being reversible and without deleterious effects for exposures of six to thirty hours' duration.

At both the higher and lower temperature levels a slight increase in tone was noticed when the experimental conditions were maintained over a period of several hours. These tone changes were always small. Amplitude changes of the heartbeat in response to

temperature variations appeared before the rhythm changes, when the temperature was either raised or lowered. Below 15° C. the amplitude gradually became reduced, and at 5° C. the height of contraction was very small, in some cases being almost negligible. Above 35° C., in most mussels, the amplitude of the heartbeat shortened even though the rate continued to increase in some cases above this temperature.

Weinland (1918) found that the oxygen consumption of the European swan-shell, *Anodonta cygnea*, during the winter months dropped to a level only one-tenth that maintained by the same animal during June, which observation implies, of course, metabolic changes varying with temperature, findings which have been confirmed by other experimenters. Since the metabolic processes of the heart must vary more or less in the same direction as the other activities of the body, and in view of the sensitivity of the heart to direct changes in temperature demonstrated in the present tests, temperature appears to be one of the dominating extrinsic factors in the regulation of the heartbeat in the fresh-water mussel.

OTHER FACTORS

When the data on heart rate of the 863 mussels were combined it was found that there is a progressive decrease in heart rate from the third to the eighth year (age determined by the ring method, Chamberlain, 1931). This decline in average heart rate is not, however, peculiar to the fresh-water mussels, as similar correlations between heart rate with age had been made for various vertebrates. In fresh-water mussels, the highest normal heart rate is found in the juvenile period but throughout this period the rate rapidly declines, and is followed by the period of maturity in which the heart rate is fairly constant. The last period is one of old age in which there is an additional senile decline in the heart rate.

The heart rate of individuals of the larger species of fresh-water mussels, in general, size for size, was slower than the heart rate of comparable individuals of the smaller species of mussels.

In Table II the actual ranges of heart rate of the various species correlated with age may be seen.

TABLE II

THE NORMAL HEART RATE AS CORRELATED WITH AGE AND SPECIES

Average Temperature 25.23°C. Range from 21.8–31°C.

SPECIES	AGE 2 YRS.		AGE 3 YRS.		AGE 4 YRS.		AGE 5 YRS.		AGE 6 YRS.		AGE 7 YRS.		AGE 8 YRS.		TOTAL NUMBER
	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Avaerge Rate	
Actionaias carinata	.....	.....	.....	.....	1	22.0	1	20.0	1	14.0	.....	.....	.....	.....	3
Amblema peruviana	.....	.....	.....	.....	.....	.....	1	20	13	14	14	18	4	11	32
Cyclonaias granifera	.....	.....	.....	.....	.....	.....	2	20	5	17	1	15	.....	.....	8
Cyprogenia irrorata	.....	.....	.....	.....	1	20	1	18	1	20	.....	.....	.....	.....	3
Elliptio crassidens	.....	.....	.....	.....	3	25	.....	.....	.....	.....	.....	.....	8	11	11
Fusconaia ebena	.....	.....	4	29	4	26	5	24	8	23	13	22	6	21	40
Lampsilis anodontoides	.....	.....	.....	.....	.....	.....	11	21	18	15	3	11	.....	.....	32
Lampsilis fallaciosa	1	34	9	34	14	28	105	23	3	26	.....	.....	.....	.....	132
Lampsilis ventricosa	.....	.....	.....	.....	.....	.....	.....	.....	1	20	.....	.....	.....	.....	1
Lasmagonia complanata	.....	.....	.....	.....	.....	.....	.....	.....	1	8	.....	.....	.....	.....	1
Leptodea fragilis	3	20	.....	.....	.....	.....	.....	.....	1	6	.....	.....	.....	.....	4
Ligumia recta	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	17	1	18	3
Megalonaias gigantea	.....	.....	.....	.....	1	20	5	16	4	16	1	16	8	10	19
Obliquaria reflexa	1	19	3	27	5	23	2	29	5	15	.....	.....	.....	.....	16
Obovaria olivaria	.....	.....	1	26	3	30	2	17	2	14	1	12	.....	.....	9
Plagiola lineolata	.....	.....	2	28	2	14	.....	.....	.....	.....	.....	.....	.....	.....	4
Plethobasus cyphus	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	18	.....	.....	2
Pleurobema coccineum	.....	.....	.....	.....	2	23	.....	.....	.....	.....	2	15	1	14	5
Pleurobema solida	.....	.....	.....	.....	6	28	6	22	15	21	8	17	5	17	40
Proptera alata	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	10	.....	.....	1
Proptera capax	.....	.....	.....	.....	.....	.....	.....	.....	1	20	.....	.....	.....	.....	1
Proptera laevissima	.....	.....	.....	.....	.....	.....	.....	.....	1	14	.....	.....	.....	.....	1

TABLE II—*Continued*

Species	Age 2 Yrs.		Age 3 Yrs.		Age 4 Yrs.		Age 5 Yrs.		Age 6 Yrs.		Age 7 Yrs.		Age 8 Yrs.		Total Number
	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	Number of Specimens	Average Rate	
Quadrula cooperiana	.....	.....	.....	.....	.....	.....	1	15	1	18	.....	.....	.....	.....	2
Quadrula cordata	.....	.....	8	29	13	24	21	21	17	14	14	14	9	12	82
Quadrula fragosa	.....	.....	.....	.....	.....	.....	1	28	1	13	.....	.....	.....	.....	2
Quadrula metanevra	2	32	2	22	11	21	42	18	55	18	8	16	4	17	124
Quadrula nodulata	3	24	1	20	.....	.....	4	19	11	21	1	21	.....	.....	20
Quadrula plena	.....	.....	.....	.....	1	24	.....	.....	.....	.....	.....	.....	.....	.....	1
Quadrula pustulosa	.....	.....	.....	.....	10	30	46	24	64	22	25	22	6	13	151
Quadrula quadrula	.....	.....	1	25	10	27	17	23	22	23	15	18	8	18	73
Quadrula subrotunda	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	14	2
Quadrula trapezoides	.....	.....	.....	.....	.....	.....	.....	.....	1	11	.....	.....	.....	.....	1
Symphynota compressa	.....	.....	.....	.....	1	18	.....	.....	.....	.....	.....	.....	1	14	2
Tritogonia verrucosa	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	28	2	15	4
Truncilla donaciformis	.....	.....	25	21	2	34	1	12	.....	.....	.....	.....	.....	.....	28
Truncilla truncata	.....	.....	1	20	.....	.....	1	17	.....	.....	.....	.....	.....	.....	2
Utterbackia imbecillus	.....	.....	.....	.....	.....	.....	1	32	.....	.....	.....	.....	.....	.....	1
Total Number of Individuals	10		57		90		276		252		113		65		863
Average Rate of Each Age	25		25		25		22		19		18		14		.....

ACTION OF SALTS ON THE FRESH-WATER MUSSEL HEART

EXTERNAL PERFUSION

Unless otherwise noted, after the mussels were opened and the apparatus attached, the perfusion was not begun until the kymograph record showed that the heart had passed into the third period of steady, regular activity (as previously described).

A mussel Ringer's fluid carrying NaCl—0.12 per cent, $CaCl_2$—0.012 per cent, KCl—0.015 per cent, and adjusted to a pH of 7.8 was found to be satisfactory for external heart perfusion and was used as the normal perfusion fluid. The records obtained from hearts perfused with this fluid were usually regular and rhythmical, and in general this salt mixture maintained the normal rhythm of the heart for long periods of time. In several cases the beat of hearts greatly fatigued, and displaying the symptoms of decline of the fourth period were restored to the normal rhythm, rate, and amplitude by perfusion with this fluid.

By varying the concentrations in the perfusate, sodium chloride was found to be the least toxic of the three Ringer's fluid salts, when these salts were present in excess. If a mussel heart were perfused with a 0.2 to 2.0 per cent sodium chloride alone for an hour or more the smooth regular rhythm of the heartbeat became irregular (Fig. 4). In some cases the rate was accelerated and in others slightly depressed by these strong sodium chloride solutions. Frequently two to five hours perfusion with strong sodium chloride solution were required for the changes produced by this salt to become apparent.

Strong calcium chloride solutions, 0.08 to 0.16 per cent (NaCl 0.153 per cent, KCl absent), depressed the heartbeat of fresh-water mussels. Calcium produced a very pronounced effect on the rate and amplitude, the depression of amplitude being particularly marked. The rhythm apparently was disturbed very little by a strong calcium perfusate. When the rhythm was regular at the start, in no case did the addition of more calcium (up to 0.16 per cent) to the perfusate produce an irregular rhythm, the rhythm tending to become more regular even though the calcium content of the perfusate were raised to 0.16 per cent. Continued perfusion of the heart with a strong calcium solution finally arrested the heartbeat in diastole.

Potassium was the most toxic of the three common blood salts, judging by the changes in activity of the mussel's heart which strong

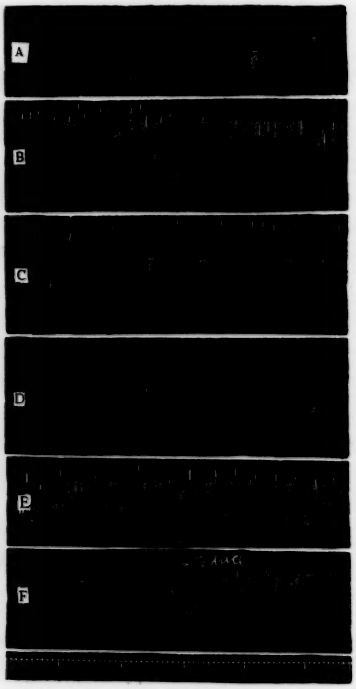

FIG. 4.—*Megalonaias gigantea*, age seven years. Temperature 25°C. Heart *in situ*, ventricular records during external perfusion, showing responses to various concentrations of sodium, potassium, and calcium salts. Time interval five seconds. *A*, Ventricular record during external perfusion with mussel Ringer's—NaCl, 0.12 per cent; KCl, 0.015 per cent; and $CaCl_2$, 0.012 per cent. Two hours and a half after opening pericardial cavity. *B*, Perfusate changed to NaCl, 0.15 per cent; KCl, 0.04 per cent; $CaCl_2$, absent. *C*, Perfusate changed to NaCl, 0.15 per cent; KCl, 0.04 per cent; $CaCl_2$, 0.08 per cent. *D*, Perfusate changed to NaCl, 0.15 per cent, KCl, 0.04 per cent, $CaCl_2$, 0.12 per cent. *E*, Perfusate changed to NaCl, 0.15 per cent; KCl, 0.08 per cent; $CaCl_2$, absent. *F*, Perfusate changed to 0.2 per cent NaCl. Both potassium and calcium absent.

concentrations of KCl induced. The depressant effect of potassium was produced more quickly and with greater intensity the larger the amount of potassium present, but a relatively strong solution of

potassium chloride was required to depress the heartbeat of the fresh-water mussel. In one case a heart perfused with 0.16 per cent KCl (sodium and calcium salts being present in normal concentrations), i.e., a concentration ten times stronger than the normal concentration of potassium salt in the Ringer's fluid used, continued to beat regularly for 88 minutes. A strong potassium perfusate affects the rhythm particularly, being the reverse of the effect of calcium which tends to maintain a regular uniform rhythm. Strong concentrations of potassium arrested the heart in diastole. Calcium antagonized potassium, and the presence of calcium mitigated the toxic effect of the sodium ion. The actions of the common blood salts on the mussel's heart are comparable, therefore, with the actions of these salts on the hearts of other animals, both vertebrate and invertebrate (see Fig. 5).

The heart of the fresh-water mussel when perfused externally with strong concentrations of the common heart salts seemed to acquire some tolerance for these salts. This phenomenon was observed with all three of the common heart salts, namely, sodium chloride, potassium chloride, and calcium chloride. When the concentration of any one of these salts was gradually increased in the perfusate and some time allowed for the heart to adjust itself before the concentration was still further increased, the final concentration of the salt in the perfusate could be carried to a higher level than if the strong concentration were applied abruptly and without time for adjustment.

INTERNAL PERFUSION OF THE HEART

The internal perfusion of the mussel heart through a fine hypodermic needle as previously described was attempted, using the Ringer's fluid which was successful for external perfusion. As an internal perfusing medium for the heart of fresh-water mussels, however, this combination of salts was not satisfactory, the heartbeat in all cases being quickly depressed to zero. Repeated tests resulted in the same responses, and no successful internal perfusion of the mussel heart was secured with this fluid. Since the Ringer's fluid used in the pericardial perfusions did not produce a satisfactory response when perfused internally, the effect of altering the salt ratios of the three major blood salts, sodium chloride, potassium chloride,

and calcium chloride, was tried in an effort to secure a successful internal perfusion fluid. It was found that for internal perfusion of the heart the ratio of potassium to calcium was too high in the mussel Ringer's as perfused externally, and that a successful internal perfusing fluid for the fresh-water mussel heart must have a greater

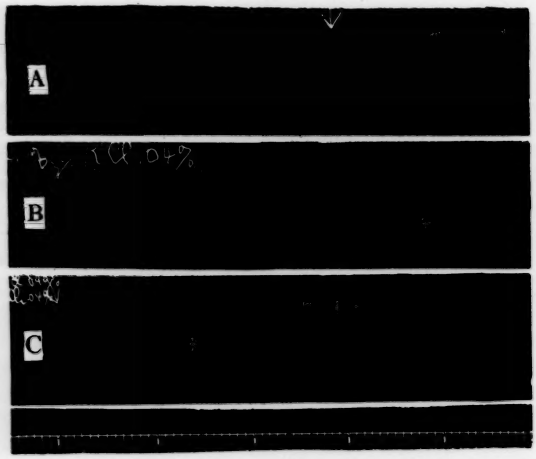

FIG. 5.—*Lampsilis anodontoides*, age five years. Temperature 25°C. Ventricular records of external perfusion with mussel Ringer's showing the effect of varying the concentration of the potassium and calcium. Time intervals five seconds. *A*, External perfusion with mussel Ringer's NaCl, 0.12 per cent; KCl, 0.015 per cent; $CaCl_2$, 0.012 per cent; two hours after opening pericardium. Perfusate at point marked by arrow changed to NaCl, 0.12 per cent; KCl, 0.015 per cent; $CaCl_2$, 0.04 per cent. *B*, Fifteen minutes later perfusate changed from second perfusate in *A* to NaCl, 0.12 per cent; KCl, 0.04 per cent; $CaCl_2$, 0.012 per cent at point marked by arrow. *C*, Fifteen minutes later second perfusate in *B* changed to NaCl, 0.12 per cent; KCl, 0.04 per cent; $CaCl_2$, 0.04 per cent at point marked by arrow. Note restoration to normal activity.

concentration of calcium than potassium and must carry a comparatively low concentration of both potassium and calcium salts. The optimum combination of these three salts for internal perfusion, as found in the experimental tests, follows, and is designated as the Internal Perfusion Ringer's; NaCl—0.12 per cent, KCl—0.003 per cent, $CaCl_2$—0.006 per cent, pH 7.5–7.8.

The limits of variations in the concentrations of calcium and

potassium tolerated by the heart when these salts were applied by internal perfusion were much more restricted than when these same salts were applied by external perfusion. If the calcium-potassium ratio were maintained at 2:1, the calcium could in most cases be varied from 0.004 per cent to 0.012 per cent, provided that the potassium were not raised above 0.004 per cent, i.e., for the higher concentrations of calcium the calcium-potassium ratio rose to 3:1. If the perfusate contained potassium in excess of the ratios given above, or were the potassium not balanced against calcium (i.e., a calcium-free perfusate) depression of the heartbeat followed rapidly. On the other hand changes in the sodium chloride content of the internal perfusion fluid were readily tolerated from 0.06 per cent to 0.12 per cent.

The extreme sensitivity of the heart to the immediate changes in salt balance produced by internal perfusion is apparently related to the internal structure of the heart. As has been pointed out previously (Motley, 1933), the bundles of cardiac muscle fibers in the fresh-water mussel heart are loosely interwoven in the walls of the auricles and ventricle; with numerous sinus-like spaces between the crossing and interlocking bundles. Inside the auricular and ventricular cavities the muscle fibers are exposed directly to the action of the fluid of the blood, there being no endocardinal lining to those cavities. Consequently the loosely bound muscle fibers of the heart wall are in an optimum position to be affected by salts or other compounds in the blood, once the blood containing these substances enters the heart cavity. The outer surfaces of the heart on the other hand, that is, the surfaces in contact with the pericardial fluid, are covered with a compact layer of epithelial cells and scattering mucous cells, so that the outside of the heart is, therefore, insulated to some extent against substances in the pericardial fluid. Hence the differences in the immediate responses of the heart to given concentrations of salt when perfused internally, as compared with the responses to these same fluids perfused externally.

In perfusing the heart of the fresh-water mussel through the ventricular wall one factor seemed outstanding in altering heartbeat, namely, dilation. Piercing the heart wall with the perfusion needle apparently did not induce inhibitory impulses depressing the

heartbeat, as in no case was the heart rate affected by the mere puncturing of the ventricular wall with the hypodermic needle, or the mechanical stimulation attendant on the perfusion adjustments. However, due to the general structure of the heart, particularly the thinness of the ventricular wall, over-dilation of the heart quickly ended all cardiac activity and such distension tended to occur unless the perfusion rate was very carefully adjusted. Two to three drops per minute through the hypodermic needle was the maximum perfusion rate tolerated by the average fresh-water mussel heart. In some hearts dilation was produced even with this amount of perfusion, regurgitation into the auricles occurring through the auriculo-ventricular valve.

EFFECT OF VARYING THE pH

Gaskell (1880) showed that alkaline and acid substances are antagonistic in action on the heart, and that the ventricle of a frog's heart stopped in diastole when perfused with weak acid and in systole when perfused with weak alkali. Hogben (1925) using *Maia*, found that the heartbeat was accelerated in an acid medium. Goljachowski (1930) demonstrated in the case of the frog's heart that the antagonism between calcium and potassium is more clearly defined the more acid the medium, and the more alkaline the medium the less pronounced the sensitivity of the heart to the lack of calcium ions.

The effects of lowering and raising the pH of the Ringer's fluid were tested on the mussel's heart by external perfusion. In one series the pH of the perfusate was decreased to 6.15, using hydrochloric acid, and in another the pH was raised to 9.0, using sodium hydroxide. The fresh-water mussel heart tolerated a larger deviation from the normal pH value of 7.8 (Ellis, Merrick, and Ellis, 1931) on the acid side than on the alkaline side. With either strongly alkaline (pH 9.5) or strongly acid (pH 5.0) condition of the perfusate the heartbeat was quickly depressed, activity ceasing in a marked systolic contraction.

Experiments were performed using the common salts of sodium, potassium, and calcium with abnormal pH values of the perfusate both above and below a pH value of 7.8. The results obtained by

varying the concentrations of these salts together with changes in the pH were very similar to the results obtained when like changes in salt concentrations were made in a solution adjusted to a pH of 7.8, and indicated no noticeable change in the permeability of the wall of the mussel heart when the pH value is shifted either up or down within the tolerated limits.

NERVOUS REGULATION OF THE HEART

Foster and Dew-Smith (1875) attacked the neurogenic theory of the heartbeat on the ground that hearts of many of the lower forms, as the gasteropods, which contract rhythmically, have no nerve cells, nor nerves running into or out of the heart at the aortic and pulmonic orifices. Yung (1881) described the hearts of *Anodonta anatina*, *Solen ensis*, and *Mya arenaria* as provided with nerve fibers from the posterior (branchial or visceral) ganglia, which nerves when stimulated accelerated the cardiac movements. Carlson (1905 *a* and *b*) states that the auricles and ventricles of the lamellibranchs in general are supplied with inhibitory nerves from the visceral ganglion or ganglia. These nerves according to that observer enter the heart at the bases of the auricles, and he infers that the molluscan heart, without exception, is provided with cardio-regulative nerves—both augmentors and inhibitors.

In the present experiments the hearts of the fresh-water mussels studied always responded to direct electrical stimulation with an increase in tone and a decrease in amplitude of beat. If the stimulation were maintained long enough and if the current were strong enough, the heart was stopped permanently. Stimulation of the visceral ganglion in these same mussels did not depress the heartbeat, although the contractions of the foot muscle induced by such stimulation often resulted in a mechanical shifting of the position of the heart which stimulated on the kymograph record an increase in tone, although there was no actual physiological change in the tone of the heart. The same condition was produced when the foot muscle was stimulated directly with the same strength of current. Stimulation of the subesophageal ganglion gave like responses. No nerves or ganglia were found in the hearts of the fresh-water mussels examined, the electrical stimulation of which would inhibit or accelerate the

heartbeat, and in a histological study of the fresh-water mussel heart (Motley, 1933) no nerve cells or ganglia were demonstrated in the walls of the auricles or ventricle in either prepared sections or by intravitam staining with Methylene blue.

To check these histological and experimental findings perfusions with three common heart drugs, atropine, adrenalin, and digitalis were made.

Concentrations of atropine sulphate (Eli Lilly & Company) varying from 1:35,000 to 1:2,000 in Ringer's fluid were used in external perfusion of the mussel heart. These concentrations of atropine produced neither stimulation nor depression of the heartbeat—tone, amplitude, rate, and rhythm all remaining unchanged. From the evidence of the experimental tests, atropine in these concentrations (which are pharmacologically potent for vertebrate hearts) exerts no influence on the heartbeat of the fresh-water mussel. These findings suggest that the mussel heart lacks a nervous mechanism comparable to the parasympathetic mechanism found in most vertebrate hearts.

Carlson (1906) found that adrenalin has a powerful stimulating action on the heart of *Limulus*, the effect being much stronger on the ganglion than on the heart muscle. Adrenalin chloride (Parke, Davis & Company) in a 1:10,000,000 dilution in unionid Ringer's may or may not produce a slight acceleration of the heart rate in fresh-water mussels by the external perfusion method. Dilutions of adrenalin from 1:2,500,000 to 1:1,125,000 produced definite acceleration of the heartbeat amounting in some cases to an increase of 7.2 per cent. Adrenalin in a 1:50,000 dilution was toxic to the fresh-water mussel heart, causing the beat to become slow and irregular. The amplitude of the heartbeat remained unaffected by adrenalin unless strong concentrations (1:50,000) were used. Concentrations of 1:50,000 or stronger produced a progressive decrease in the height of contraction. Figure 6 shows the action of different dilutions of adrenalin. The low sensitivity of the fresh-water mussel heart to adrenalin is in accord with the atropine experiments. As adrenalin acts both on muscle fibers and on sympathetic nerve mechanisms the slight response of the fresh-water mussel heart to adrenalin could readily be explained as one of direct action on the muscle fibers them-

selves, since the concentrations of adrenalin used on the mussel heart, if applied to a vertebrate heart possessing a sympathetic mechanism, would have produced responses many times more profound.

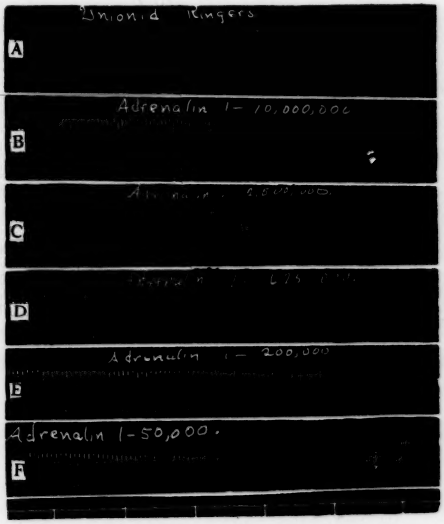

FIG. 6.—*Amblema peruviana*, age six years. Temperature 25.6°C. Heart *in situ*, ventricular records during external perfusion showing responses to various concentrations of adrenalin chloride in mussel Ringer's. Time interval one minute. *A*, External perfusion with mussel Ringer's twelve hours after opening pericardium. *B*, Fifteen minutes later, perfusion with Ringer's carrying 1:10,000,000 adrenalin chloride. *C*, Fifteen minutes later, adrenalin concentration 1:2,500,000. *D*, Fifteen minutes later, adrenalin concentration 1:625,000. *E*, Fifteen minutes later, adrenalin concentration 1:200,000. *F*, Fifteen minutes later adrenalin concentration 1:50,000, producing periods of heart standstill in diastole. Note prompt return of heartbeat after change in Ringer's fluid without adrenalin at point marked by arrow.

A 0.5 per cent solution of digitalis (E. R. Squibb & Son) in mussel Ringer's strengthened the heartbeat of the fresh-water mussel when applied by external perfusion. The individual contractions of the heart were more powerful, although there was a tendency toward incomplete relaxation. External perfusion of digitalis maintained a regular uniform rhythm of the heartbeat for a long period of time. In

several cases hearts which were beating irregularly and with slight amplitude, as a result of fatigue from long exposure, were restored to regular uniform rhythm with an increase in the height of contraction by perfusion with digitalis. Strong concentrations of digitalis, as 1.0 per cent, suppressed the heartbeat in systole. In the main the action of digitalis on the fresh-water mussel heart was comparable to the action of this drug on vertebrate hearts, in which digitalis is known to affect heart-muscle, although the actual quantity of digitalis required to produce these effects in the fresh-water mussel heart was much larger than the doses required for vertebrates.

In the present work, therefore, no evidence was secured suggesting any nervous regulation of the fresh-water mussel heart.

DISCUSSION

Reviewing all of the observations collectively, the heartbeat of the fresh-water mussel was found to be very sensitive to sudden changes in the chemical composition of the blood actually passing through the heart, primarily because of the absence of an endocardium, the cardiac muscle fibers being in direct contact with the fluid contained in the auricular and ventricular cavities. On the other hand, if the changes in salt balance and pH were made gradually the heart of the fresh-water mussel could adapt itself rather rapidly over proportionally much wider ranges of pH and of salt concentration than the average vertebrate heart. The ability of the fresh-water mussel to close its valves quickly and remain alive without asphyxia for a period of hours or even days, using only the water held in the mantle and branchial cavities, represents a protective characteristic which allows the heart to make the gradual adjustments to the changes in salt balance and chemical composition of the surrounding water for it was been demonstrated by Ellis, Merrick, and Ellis (1931) that the blood of the fresh-water mussel rapidly takes up salts from the surrounding water if the valves of the mussel be propped open and the closure of the mantle cavity prevented.

The reactions of the fresh-water mussel heart under some conditions were characteristic of smooth muscle, as the heart operated best under the stimulus of slight distension and a normal internal pressure. Gaskell (1880) thought a certain amount of internal pres-

sure necessary to produce automatic contractions in the heart of Mollusca, and Ransom (1884) stated that the two organs, the auricles and ventricle, in the heart of cephalopods are physiologically isolated. Skramlik (1929) concluded that there are no automatic centers in the hearts of mollusks and that the ventricle is of primary importance in the maintenance of the circulation as the filling of that part of the heart with blood stimulates the heart to activity. The present findings on the heartbeat of the fresh-water mussel agree quite closely with Skramlik's general observations, and with the more recent findings of Heinbecker (1933) on the heart of *Limulus*. The ventricle was found to hold the important part in the maintenance of circulation, a slight tension on this structure and a certain amount of pressure within the ventricle facilitating the automatic contractions, the ventricle being much less dependent on the auricle in the fresh-water mussel heart than in vertebrate hearts.

SUMMARY

The regulation of cardiac activity in the fresh-water mussel seems to depend largely on the chemical composition of the fluid actually passing through the heart, the internal pressure of that fluid on the auricular and ventricular walls, and the body temperature of the animal. If changes in salt content and pH of the fluid passing through the heart are not made too rapidly the fresh-water mussel heart can adapt itself to proportionally wider variations in salt content and pH than can the average vertebrate heart. Nervous control is apparently negligible or of very little consequence in the major adjustments of heart activity. Correlations of lesser importance with age, species-size, and species-activity were also found.

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EXPERIMENTS IN GROUP BEHAVIOR OF FISHES

(Eleven figures)

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BACKGROUND LITERATURE[1]

FISHES were not credited with an ability to learn through experience until Möbius (1873*b*) performed his much-quoted experiment. Subsequent and increasingly critical experiments have shown that fishes are capable of considerable learning. In verifying Möbius' work, Triplett (1901) placed two perch in one end of an aquarium and separated them from minnows in the other end by a glass partition. After repeatedly dashing against the partition to catch the minnows the perch became so thoroughly conditioned against them that they would not rush toward the minnows even though the partition were removed and the minnows swam about them. However, if worms were dropped on the other side of the partition the perch again dashed violently against it in their attempts to eat. This showed that they had learned to avoid the minnows and not the partition, and that they had not merely become fatigued. This conditioned-response technique has been fruitfully used in most of the subsequent experiments in the behavior of fishes.

Thorndike (1899), Goldsmith (1905, 1914) and Churchill (1916) trained fishes to run mazes and showed that both sight and "motor memory" played a part in the conditioning process. Experiments on color vision in fishes proved that they can discriminate a wide range of colors: Zolonitsky (1901), Washburn and Bentley (1906), Reighard (1908), von Frisch (1912), Goldsmith (1914), Schaller (1926), and Bull (1928). That fishes can discriminate wave lengths and not merely intensities of light was definitely shown by White's (1919, 1927) work with mudminnows and sticklebacks.

[1] Not only for suggesting this project but also for his helpful interest in it throughout, I wish to express my deep gratitude to Dr. W. C. Allee. In part this work was aided through a grant to the University of Chicago from the Rockefeller Foundation.

Fishes are easily and quickly trained to form motor responses to sound vibrations: McDonald (1922), Westerfield (1922), von Frisch (1923*b*), and Bull (1928). Stetter (1929) found fishes had an auditory range of over five octaves and that their auditory acuity compared favorably with man's.

Further discriminatory capacities of fishes have been discovered through recent work. Bull found that the blenny could form conditioned responses to slight changes in temperature or salinity of the surrounding water. Striek (1924) discovered that blinded *Phoxinus* could discriminate between grape sugar, acetic acid, and quinine even after extirpation of the forebrain. Trudel (1929) trained minnows to recognize various sweet substances such as glucose and proved their taste sense keener than man's.

Ample demonstration exists that fishes can remember. Experiments by several workers have established the retention of conditioned behavior for the following periods: Churchill—13 days; Goldsmith (1914)—18 days; Reighard—20 days; Russell (1931)—98 days.

Very stimulating results have been recently obtained by configurational or *Gestalt* psychologists. The work of Schaller (1926), Herter (1929), Perkins and Wheeler (1930) and Perkins (1931) introduces the conception that fishes can recognize and react to abstract relationships—that they can grasp "configurations." The fishes in question were trained to react to such things as geometric figures of different sizes and colors or to relative light intensities. The experimenters ascribe this learning behavior to "insight" rather than to trial and error.

As to conditioned behavior of groups of animals generally, very little experimental work has been done on animals other than man. The great difficulty of establishing adequate controls for human subjects seems to be largely responsible for the widely disagreeing results obtained in human group experiments. Many such experiments have shown decided influences of competition, pace-setting, stage-fright and so on. Hudelson's study (1928) of the effect of class size on university instruction indicates, contrary to popular notion, that large university classes do not tend to lower teaching efficiency. Of 21 similar investigations summarized by Hudelson,

14 show no, or dubious, correlation between class size and efficiency, 5 show positive correlation, and 2 negative.

There is no question that a group of animals influences the individuals in it. Alverdes (1927) indicates that the young of animals and children eat more in groups than when isolated; that man and beast sing more vigorously in concert. Morgan (1900) tells of a mongrel pup whose I.Q. was boosted through association with dogs of superior intelligence. Experimenting with chicks he found that "If a group of chicks have learnt to avoid cinnabar caterpillars, and if then two or three from another group are introduced and begin to pick up the caterpillars, the others will sometimes again seize them, though they would otherwise have left them untouched." The writer has seen a similar influence working between two angel fishes (*Pterophyllum scalare*). One fish fed to satiety will refuse angleworms falling near it until the other fish attempts to snatch them. Often, in such a case, the first fish will then seize the worm and eat it.

Several observations have been made on apparent synchrony of rhythmic behavior in animals; these are adequately reviewed by Allee (1931). They show a widespread tendency toward group unity.

Studies on imitation in animals constitute a special approach to the field of group behavior. Clear evidence of imitation is found in monkeys (*Cebus* and *Macacus*) by Kinnaman (1902) and Haggerty (1909). The same imitative tendency is found in white rats and cats though not so pronounced (Berry, 1906, 1908). Conradi (1905) tells of the metamorphosis of the songs of English sparrows when reared with canaries.

The possibilities of communication between animals are largely unexplored. The experiments of von Frisch (1923*a*) with honeybees revealed that a marked bee that had discovered a watched source of pollen or nectar communicated this information to other bees within the hive by means of a kind of whirling dance; the dance varied according as the booty discovered were nectar or pollen.

The experimental study of group behavior in fishes is a new field. Parr's study (1927) of schooling behavior in the chub mackerel showed that visual stimuli provided the integrating factor. A fish temporarily blinded will neither school nor mill with others in the same aquarium. Bowen (1931, 1933) has shown that visual and

thigmotactic responses were involved in the aggregating behavior of young catfishes (*Ameiurus melas*). Experiments done in this laboratory by Henderson, Miller, and Welty (all unpublished) have indicated an inhibitory influence of the group in conditioning the behavior of mudminnows. Because the author's experiment was introductory to those that follow in this paper, it is briefly reported herewith.

MUDMINNOWS AND GROUP INHIBITION

Experiment 1.—Mudminnows (*Umbra limi*) were distributed as follows in large (see Materials and Methods) aquaria the four sides of which were covered with black paper: one each in five tanks, two each in two tanks, four each in four tanks, and ten each in two tanks. Each tank held the same amount of aerated water. The fishes were conditioned to jump out of water for food held on the end of a feeding needle (see below) when a redlight showed 75 mm. overhead. They were also trained to refuse the food (dipped in acetic acid or composed of bits of rubber tubing) when exposed to a green light of equal intensity. The results at the end of 48 days of trials were inconclusive. No differential learning rates could be detected with certainty. When, however, the results were studied on a basis of jumps per fish per minute of exposure there seemed to be a slowing down in the larger groups. Whereas the isolated and also the paired fishes averaged about 20 jumps per fish per minute under the red light, the groups of four averaged 10.3 and groups of ten, 5.9 for the 48 days.

While a part of the slowing down of the groups was undoubtedly due to the lessened opportunity of jumping in a given time—two fish rarely jumped at once and three never did so—observations showed a definite antagonism between members of a group which prevented many otherwise possible jumps. As one fish would assume the characteristic jumping position another fish would often attack it with a vigorous jab in the belly. These results are mainly reported here because of their apparent disagreement with the general findings of later experiments.

MATERIALS AND GENERAL METHODS

The experiments that follow were performed between September, 1929, and September, 1932, at Whitman Laboratory of Experimen-

tal Zoölogy, University of Chicago; at the Marine Biological Laboratory, Woods Hole, Massachusetts; and at Parsons College, Iowa. Except where stated otherwise they were performed at Whitman Laboratory. About 930 fishes were used, most of them goldfishes, *Carassius auratus*. The other species used were: the Pursy Minnow *Cyprinodon variegatus*, the Killifish *Fundulus heteroclitus*, the Lake Shiner *Notropis atherinoides*, the Paradise *Macropodus opercularis*, and the Zebra Fish *Brachydanio rerio*.

Goldfishes were used for most of the experiments because they were available in quantities throughout the year, hardy under laboratory conditions, and relatively active. Except in the case of *Fundulus*, sex was not determined; the goldfishes were immature, about 50 mm. long. Unless specific mention to the contrary is made below, all fishes were permanently discarded at the end of each experiment. To distinguish individuals in groups fishes were first marked with india ink injections (Keys, 1928) but it was found that the spots thus made faded in a month or two, so a system of clipping off bits of the caudal fin was substituted. In case a fish died during an experiment its record was used only for that period during which it appeared to be in active good health.

Temperature and light were not controlled excepting as the experiments were housed in laboratory rooms or in a greenhouse with steam heat under rough thermostatic control. Some of the early experiments were performed either in a dark room or at night so that a red-light stimulus might be used. It was found, however, that the light was either so bright that it apparently dazzled the fishes, or else so dim that the experimenter had difficulty distinguishing them. Furthermore the fishes appeared to be less active at night, or in the dark, and hence became conditioned less rapidly, so most of the experiments were carried on by daylight. A control experiment with the red-light stimulus showed neither positive nor negative phototropism.

Ordinary laboratory aquaria were used for most of the experiments. They were constructed of galvanized and painted angle-iron frames into which were cemented double-thickness sheets of window glass. The *large* aquaria used measured 26.5 cm. high, 26.5 cm. wide, and 30.5 cm. long, outside dimensions. *Small* tanks were 20.5 cm.

high, 22 cm. wide, and 25.5 cm. long. City tap water was used except in the experiments with marine species where running sea water was employed. Ordinarily the water was 18 cm. deep in large tanks and 15 cm. in small. Tanks were always cleaned between experiments and sometimes sterilized when fungus growth of parasites threatened. For the first experiments bubbling air, and in one instance small pots of *Vallisneria* were used to oxygenate the water, but this was later found unnecessary and was discontinued.

EXPERIMENTAL PROCEDURE

As the experiments progressed both the materials used and the experimental technique became considerably modified so that a general description of a typical experiment becomes difficult. This unfortunately makes necessary the inclusion of much descriptive matter. The method most generally used consisted essentially in offering a stimulus at one end of the aquarium to which the fish responded, when conditioned, by swimming (toward the stimulus, e.g.) from the other end of the tank through an opening in a barrier. The procedure followed in Experiment 2 will serve as an introduction to the remaining details of materials and methods as used in the early experiments. Modifications of procedure and apparatus will be explained later as necessary.

Experiment 2.—A battery of seven large aquaria was arranged on a waist-high shelf in a dark room. Each tank was filled with tap water to a depth of 18 cm. The front end (toward the experimenter) of each tank was covered with a removable piece of cardboard, the remaining three sides were permanently covered with black paper. Each tank was supplied with bubbling air. Into each of the seven tanks was inserted a screen barrier composed of one-eighth inch mesh galvanized wire (Fig. 1). It extended the full width of the tank and from the top to the bottom, located parallel to and 10 cm. from the front end of the tank. In the center of the screen was a conical opening that led from the rear chamber to the front one; the chambers were created by the screen. The cone gate or entrance was .65 cm. long with a circular opening 7.5 cm. in diameter where it fastened to the screen barrier and an opening 2.8 cm. in diameter at the smaller front end. A sliding metal plate controlled this opening.

Unselected goldfishes averaging 45 mm. in length (always excluding the tail-fin) were distributed in the tanks as follows: one fish in each of the first four tanks, two in each of the next two tanks, and four fishes in the last tank. The fishes were allowed to become accustomed to the tanks with the gates left open for seven days. Then those that happened to be in the front compartment were gently

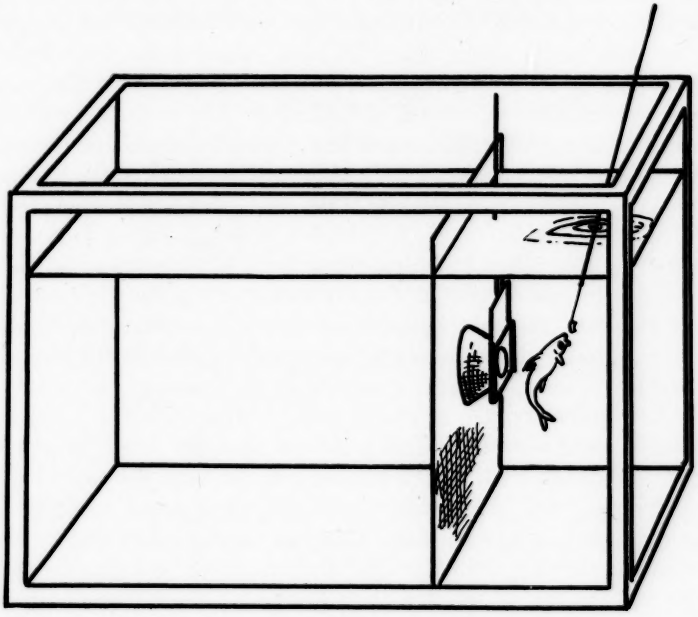

FIG. 1.—Cone-screen maze used in Experiments 2 to 8

maneuvered into the rear compartment and the gates were closed. On the ninth day the first trials were run as follows. The red light used in Experiment 1 was so placed that it shone directly into the center of the cardboard covering the front of the tank. Then the gate of the screen was opened and the cardboard removed simultaneously. Eventually the fish or fishes in the tank found their way through the opening by random movements. As soon as a fish passed through the cone-gate it was fed under water with a bit of

freshly cut earthworm impaled on the end of a feeding needle. This needle was composed of a stiff wire handle 23 cm. long and 1.2 mm. in diameter with a fine German silver bristle 5.8 cm. long and 0.3 mm. in diameter soldered to the handle. The meat was affixed so that it was easily removed. Where several fishes were in a tank the gate was left open and the light on until all the fishes had come through the gate and were fed. In group tanks it often happened that one or more fishes would swim back and forth through the gate after their initial performance. In such instances the fish was fed on its first performance but never on subsequent ones of that trial. The needles allowed controlled feeding so that each fish was fed only once in a given trial. A fish was arbitrarily considered through the opening when its dorsal fin had passed the plane of the circular opening at the outer smaller end of the cone. Fishes so located rarely if ever turned back. After all the fishes in a tank (or *the* fish in the case of isolated fishes) had come through the gate and were fed, they were gently maneuvered under the raised screen barrier into the rear chamber, the light was shut off, and the cardboard front and screen replaced. The screen was moved only at the end of a trial for returning fishes to the rear compartments. The fishes were never handled either between or during trials. After trials the tanks remained closed and in the dark until the next day when the above routine was repeated. Daily trials continued until the end of the experiment. The data obtained from this experiment consisted of the reaction time of each fish. This was the time that elapsed between the turning on of the light and opening of the gate in each tank and the time at which a given fish passed through the opening and was fed. The reaction time of fishes in groups was compared with that of isolated fishes on this basis.

GROUP LEARNING *vs.* ISOLATED LEARNING

Experiment 2.—The procedure followed in this experiment has just been given. A goldfish in the group of four died on the day of the third trial and its record was not used in determining means. A substitute fish was placed with the three survivors and its individual record figured in average computations only from the fifth trial on. To serve as a check on this group an auxiliary group of four fishes

in a tank was started on the thirteenth day (trial) of the experiment. The mean reaction times are given according to type of grouping in Table I. In all cases "mean reaction time" means the average reaction time of all fishes of a given class on a given trial. The ineffectiveness of continued conditioning after the sixth or seventh trial is especially noticeable. While the mean reaction time for the fishes in the auxiliary group of four shows superior speed of learning, the data from the original set-up furnish no evidence of a marked group influence.

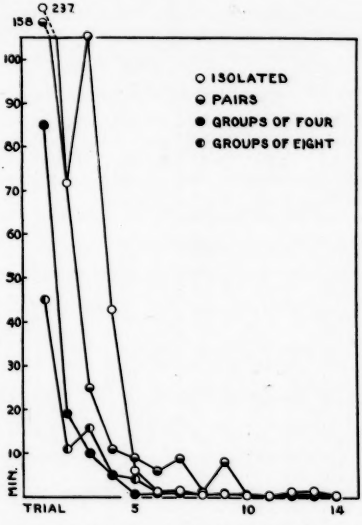

FIG. 2.—Mean reaction time for gold fishes in a cone-screen maze. Experiment 4

Experiment 3.—In this repetition of Experiment 2 the following modifications were introduced. The aquaria were arranged on a shelf in a greenhouse and cardboard covers were placed over them (in addition to the usual side-coverings) to eliminate algal growth. The red-light stimulus was made brighter. There were 15 tanks into which the fishes were distributed thus: one each in the first eight, two each in the next four, four each in the next two tanks, and eight in the last tank. Other conditions were as before. The brighter light seemed to blind the fish as they did not take the worm fragments

from the feeding needles immediately upon performing as they did before. No certain influence of the group is evident (Table I).

Experiment 4.—Fifteen aquaria with screens as before were set up in the greenhouse and the red-light stimulus discarded for daylight. Fish, distributed as in Experiment 3, were given a nine-day acclimation period in the tanks with the gates open. Trials were run daily. Small pots of *Vallisneria* were placed in the right rear of each tank

TABLE I

MEAN REACTION TIME OF GOLDFISHES IN A CONE-GATE MAZE

(Time is given in minutes and hundredths)

Experiment Number	No. of Fish per Tank	Trial Number							
		1	2	3	4	5	6	7	8
2.	1	26.30	5.05	7.78	1.47	0.97	1.17	0.76	0.43
	2	38.41	13.34	16.84	3.99	1.75	0.58	1.22	2.81
	4	18.07	19.09	6.00	2.09	2.08	0.47	0.53	0.72
	4*	5.25	2.95	0.76	1.59	0.68	0.25	0.30	0.34
3.	1	88.84	18.51	13.97	7.49	6.28	3.18	7.42	4.09
	2	83.14	12.14	13.88	8.36	6.38	5.07	6.16	4.49
	4	132.00	16.97	7.83	6.58	3.74	3.99	6.78	3.75
	8	18.30	16.03	9.01	7.25	3.70	5.33	2.43	5.34
4.	1	237.00	71.61	105.23	43.16	5.92	1.22	1.41	0.45
	2	158.00	73.00	25.34	11.03	8.76	5.95	8.91	1.28
	4	85.00	19.09	9.95	4.92	0.63	0.91	0.74	0.55
	8	45.25	10.50	15.68	4.28	4.17	1.53	1.68	1.61
5.	1	284.00	118.33	44.26	38.75	19.00	3.26	10.50	2.76
	2	214.41	121.85	6.57	1.49	1.28	0.64	0.26	0.28
	4	13.50	14.17	4.58	2.01	1.42	0.26	0.22	0.36
	8	17.42	4.68	2.32	1.22	0.32	0.30	0.25	0.28

* Auxiliary group.

for aeration. The records of two of the isolated fishes were deleted because the fishes were refractory; they did not respond successfully within eight hours of presentation time on the first trials. The results of this experiment reveal a pronounced group influence; the larger the group the quicker the conditioning. Data are given for the first eight days in Table I and shown graphically for the first fourteen days in Figure 2.

Experiment 5.—This was a repetition of Experiment 4 with two minor modifications. Bubbling air was used for aeration. Instead

of giving daily trials in the same tank-order—1, 2, 3, 4, ... 15—each day, the order was now reversed daily: 1 to 15 one day and 15 to 1 the next. The results obtained corroborate those of Experiment 4; the larger the group the quicker the conditioning. A fish in the group of eight died on the day of the fourth trial; its record was ignored in final computations. Three fishes were mainly responsible for the greatly retarded mean reaction time of the isolated fishes. This same experiment continued on as another experiment in retention and the graph is shown in that connection in Figure 6. Data for this experiment are given in Table I.

INFLUENCE OF LEADERSHIP

Experiment 6.—This experiment was really run as two separate preliminary experiments to see what effect a trained fish might have upon its untrained fellows. In addition to the fifteen tanks mentioned above, two other similar tanks were arranged in the row each time in Experiments 4 and 5. Into these tanks (Nos. 16 and 17) were placed one and two previously trained fishes respectively. Also, with these trained fishes were placed new untrained fishes, one in each tank. These extra fishes were treated along with those in the regular experiments and in exactly the same fashion. A decided influence of leadership was apparent in the results. The untrained goldfishes in both types of tanks (tanks with one and with two trained fishes respectively) learned successful responses in about one-half the mean time for the groups of eight. Those fishes with two trained leaders each learned in less time than did those with one each.

Experiment 7.—In order to check the results of these preliminary experiments on leadership and to analyze the nature of leadership, a new experiment was arranged. Fifteen aquaria were placed in a row on a greenhouse bench with screens, water, air, black coverings, and cardboard fronts as before. Seventy-four goldfishes, after being marked, were distributed thus: Into the rear (larger) compartment of each tank were placed four unconditioned fishes. Into the *front* chamber of tanks 2, 5, 8, 11, and 14 was placed an unconditioned fish each. Into the *rear* chamber of tanks 3, 6, 9, and 12, along with the four unconditioned fishes, there was placed a trained fish each.

These latter fishes were obtained from tank 15 of the group-learning experiment (No. 5) which had ended but two days previously. All other fishes were from the same new stock and averaged 41 mm. in length. During a two-week acclimation period the gates were kept closed. The object of the experiment was to determine what influence might be exerted by a leader or conditioned fish that lives and reacts with the unconditioned group as compared with the influence of a fish already through the barrier that acts merely as a visual stimulus or a lure. The tanks that contained four unconditioned fishes only served as controls. The untrained "lure" fishes stayed in front of the barrier screen both during and between trials whereas the conditioned leaders performed with their untrained fellows each day—generally ahead of them of course. The leader was always fed as soon as it performed; the lure fish was fed only after the first untrained fish in its tank had reacted and was fed. The removal of the front cardboard with the subsequent penetration of daylight into the tank, and the opening of the gate of the screen served together as the stimulus. While in these daylight-stimulus experiments the fishes were probably able to see the experimenter, great caution was exercised to prevent movements that might act as stimuli that would either enhance or inhibit reactions of fishes. The results of this experiment (Fig. 3*A*) seem to indicate that leadership stimulates learning and that it has an influence over and above that of a mere lure. The reaction time of the leaders is of course not included in computations.

Experiment 8.—Well-trained fishes from Experiment 7 were used as leaders in this experiment. All other fishes were new. After a fourteen-day acclimation period the trials were run as before. The results, given in Figure 3*B*, show a decided influence of leadership. Excepting the records for the fifth trial on which the mean reaction times of the two were nearly identical, the group-plus-leader fishes reacted in less time than the controls (and also the group-plus-lures) on each of the first eight trials; the curves reveal that these are the significant trials. This implies that the training significance of the leaders depends not merely on their presence on the opposite (front) side of the barrier but on their total reaction.

Experiment 9.—Three major modifications were introduced in this

experiment. First, the fishes were grouped differently. An objection may be raised against the variation in group size in the two previous experiments. The groups of experimental fishes contained a total of five fishes (four plus a leader or a lure) and the controls contained only four each. In the present experiment there were but two varieties of groups—control groups of three unconditioned fishes each and experimental groups of two unconditioned fishes plus one conditioned fish. There was no acclimation period; the fishes were given their first trial about half an hour after being placed in the

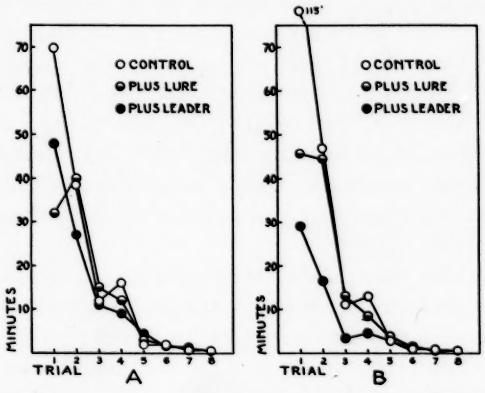

FIG. 3.—Mean reaction time of goldfishes in a cone-screen maze showing influence of leadership. *A*, Experiment 7; *B*, Experiment 8.

aquaria. A third modification was the substitution of a flat-gate screen for the cone-gate screens. Being bottom feeders the goldfish found the bottom opening in the new screens by random movements in less than the time customary with the elevated gates. This type of screen was used later in group-cohesion experiments and is illustrated in Figure 4. In the present experiment the screen barriers were used singly in each tank. They were composed of one-eighth inch wire screen and were placed parallel to and 7.5 cm. from the front end of small tanks. In the lower right-hand corner of each screen was placed a 5×5 cm. square opening with a metal sliding door as before. The water in each tank was kept at a depth of 15 cm. The fishes averaged 45 mm. in body length. The results confirm

those of the two previous experiments. The mean reaction time of the control fishes was more than treble that of the experimentals on each of the first five days.

Several attempts were made to find a statistically reliable method for determining the significance of differences in the so-called learning curves. The difficulties involved seem largely due to our ignorance of what learning is. It is impossible to ascribe the difference between two given curves to difference in mean learning ability. Other variables than that of learning are involved. First, the motivations provided by the original conditions of the experiment undoubtedly drew forth variable mean responses from the contrasted groups. A second variable resulted from the varying mean capacities of the two types of groups to learn the required behavior; this by virtue of direct influence of type of grouping, and indirect influence of variable motivation, on learning rate. Third, it is probable that the contrasted types possessed variable mean abilities to retain what they learned. Mean reaction times depended on all these influences and possibly others.

However, even though one cannot distinguish how much of the difference in performance between the experimentals and controls is due to learning, one can figure whether or not the daily difference between the mean reaction times of the contrasted groups possesses statistical significance. Student's (1925) method was used in computing the statistical probabilities in Table II. The figures represent the number of chances, on a basis of one, that random sampling would give as great a variation in behavior.

A more significant view of the reality of the differences in conditioned behavior between the contrasted groups can be had by smoothing and weighting curves of several similar experiments. This method is contrived to bring out the significance of those portions of the curves which should be most expressive of difference in learning rates.

Obviously the mean results of the first trial in any experiment reveal nothing about learning. Differential learning rates, if any, should show up with progressive significance from the second trial on. But since both groups, experimental and control, eventually reach the same efficiency of performance, i.e., their curves flatten out

together—any such progressive difference between them is obliterated. One must therefore look about midway between the first trial of any experiment and that trial on which the two curves later coincide for an expression of the most significant difference between the two curves.

The mean reaction times for the contrasted classes (isolated versus groups of four in this case) for the first five days of a given experiment were determined. These mean reaction times were then weighted so as to emphasize that period during which the curves should show the most significance in learning divergence. The mean

TABLE II

STATISTICAL SIGNIFICANCE OF DIFFERENCES IN REACTION TIME OF FISHES IN LEADERSHIP EXPERIMENTS FIGURED ON A DAY-BY-DAY BASIS

Experiment	Significance between Reaction Time of:	Day				
		1	2	3	4	5
7.....	Plus leaders and controls	0.150	0.336	0.623	0.046	0.622
7.....	Plus leaders and plus lures	−0.106	0.844	0.151	0.434	−0.921
8.....	Plus leaders and controls	*0.0000	*0.011	*0.020	*0.022	0.921
8.....	Plus leaders and plus lures	0.209	*0.022	*0.012	0.127	0.844
9.....	Plus leaders and controls	0.154	*0.0004	*0.0003	*0.007	*0.013

* Statistically significant.

reaction time for the isolated fishes on the first day (in a given experiment) was unweighted; that of the second day was doubled; that of the third day was trebled; that of the fourth day was doubled; that of the fifth day was unweighted. These values were then added. The same weighting applied to the compared groups of four so that two figures finally represented the performances of the two contrasted groups for the first five days of that particular experiment. Such pairs of figures were obtained for experiments 2, 3, 4, 5, and also 16, 17, and 18. Applying Student's method to these seven pairs of figures gave probabilities of 0.020 which indicate statistical significance. The significance does not apply to difference in learning ability directly but is very possibly an oblique indication of its existence.

For the remaining experiments significance will be read directly from the tabular data or from the graphs.

GROUP-COHESION EXPERIMENTS

These experiments were designed to reveal what part, if any, a possible group-cohesive force might play in the conditioning of fish behavior. The groups of control fishes were trained to choose one of

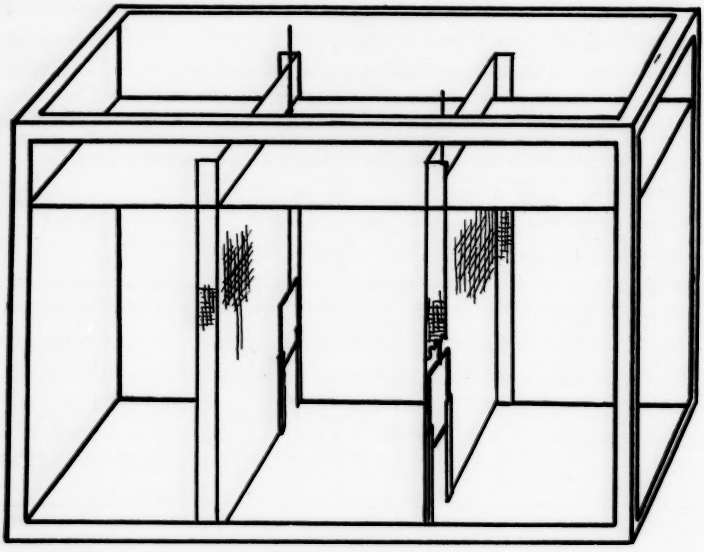

FIG. 4.—Simple maze for studying group cohesion. Experiments 10 to 15

two doors in a simple maze whereas the experimental fishes were conditioned to choose both of the two doors; i.e., certain marked fishes in the group always chose one door and the rest in that group always chose the other door. The control groups reacted intact; the experimental groups had to split. Figure 4 shows the type of maze used. At the start of the trials both the experimental and control groups were placed in the central compartment of each tank. After the gates were opened at each trial, the fishes by random movements eventually found their way through one gate or the other. If a given fish went through the gate which the experimenter had arbitrarily

selected as the correct gate it was fed. Otherwise not. Both gates were left open on every trial until the last fish of each group had performed successfully and was fed. The groups were then gently driven to the center compartment and the gates were closed until the next (daily) trial. The "correct" gate for a given fish remained the same throughout the experiment. Since different sized aquaria and different stimuli were used, further details will be given under specific experiments.

Experiment 10 (Marine Biological Laboratory).—Aquaria 49 cm. long, 35 cm. wide, and 33 cm. deep with cypress ends and bottoms were used; running sea water was kept at a depth of 32 cm. throughout the experiment. Two screen barriers were so placed as to divide each tank into three equal-sized compartments. (See Fig. 4.) Gates were placed in the bottom of each screen as before; the openings were 5 cm. square and were set in 2 cm. from the edge of the tank—the front gate on the lower left-hand side, the rear on the right. The experiment was conducted in a dark room. A 60-watt Mazda lamp with reflector was hung 35 cm. over the center of each tank to serve as a stimulus during trials. The tanks were so sheltered that only the fishes being given their trial were illuminated.

Sixteen killifish (*Fundulus heteroclitus*) averaging 70 mm. long were marked with India-ink injections and distributed four each in four aquaria. In the first tank all four fishes were rewarded only when they came through the gate toward the front of the tank; in tank two, fishes A and B performed successfully by coming through the front gate and C and D by passing through the rear gate; in tank three all four fishes were trained to pass through the rear gate; in tank four the group split as in tank two. Tank two contained exclusively females, the other tanks contained only males. The fishes in each tank were allowed a two-day acclimation period with gates open. The results showed no significant difference in behavior between the contrasted groups.

Experiment 11 (Marine Biological Laboratory).—This was a repetition of the preceding experiment using, however, *Cyprinodon variegatus* instead of *Fundulus*, and clipping tails for identification instead of using ink spots, which became infected with a luminescent mold or bacterium. The results of this experiment showed a definite separa-

tion of the two mean reaction time curves (Fig. 5). The cohering groups apparently learned in less time than the separating groups.

Experiment 12 (Parsons College).—Large aquaria were fitted with screens similar to those in Experiment 11 so as to divide the tanks into thirds. The gates were 5×5 cm. The stimulus was a red light in a wooden box presented at the front end of a given tank during trials. All trials were run at night. Goldfishes averaging 40 mm. in length were used; their fins were clipped for individual identification as before. Results for this experiment showed no significant difference between the cohering and the splitting groups in rates of condi-

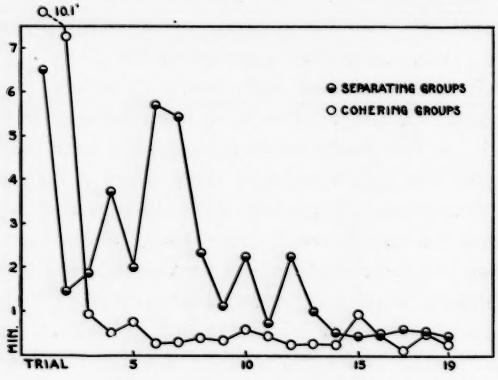

FIG. 5.—Mean reaction times for *Cyprinodon* in group-cohesion experiment

tioning. The behavior of the fishes was probably influenced through their being exposed to an active laboratory environment between trials.

Experiment 13.—Eight large aquaria were each divided into three equal chambers by screen barriers as before. The gates were 5×5 cm. square and were set in 12 mm. from the edges of the bottoms of the screens. The front gates were to the experimenter's left and the rear to his right. As before, all tanks were covered with black paper on the two sides and the rear; the front was covered with a removable card board. This experiment and all subsequent ones reported here were performed at Whitman Laboratory. A red light placed against the front glass of each tank (in turn) served as a stimulus.

The four marked gold fish in each tank were given a three-day acclimation period with gates open. Daily trials continued for 37 days; the fishes were then transferred to another experiment on the effect of feeding which will be treated below. Fishes in half of the tanks separated for a successful performance while those in the remainder of the tanks were trained to pass through only one of the possible two openings. Excepting for trials 1 and 2, the cohering groups reacted successfully in less time than did the splitting groups until the twelfth trial; from then on both groups performed about equally well. This indicates that group cohesion was a factor in stimulating the conditioned behavior of these fishes.

Experiment 14.—In this attempt to discover the rôle of cohesion in group-learning three modifications were introduced. Small tanks were used and daylight provided the stimulus. The split groups were conditioned to divide one to three instead of two to two. The cohering groups reacted as formerly. During this experiment, which ran 18 days, the excessive summer heat caused so many fishes to die that the records of half of the tanks had to be thrown completely out. The remaining data showed no cohesion advantage.

Experiment 15.—This was essentially a repetition of Experiment 14 with one variation: three 45 mm. goldfishes were placed in each small tank instead of four. In the separating groups one fish reacted in an opposite direction from the remaining two. Possibly due to records of refractory fishes in tank 1, no significant differences in the mean reaction time curves of the two types of groups were found.

EXPERIMENTS IN RETENTION

These experiments were set up to determine whether the grouping of fishes had anything to do with the retention of conditioned behavior. Goldfishes approximately 45 mm. in body length were used throughout and all experiments were performed in the Whitman Laboratory greenhouses.

RETENTION THROUGH TIME

Experiment 16.—An attempt was made to determine not only whether grouping influenced retention but whether the influence, if any, came from learning as a group, from retaining as a group, or from both. Thirty-two goldfishes were distributed in twenty small

tanks thus: tanks 5, 10, 15, and 20 contained four fishes; each of the other tanks, one. In each tank was placed a single flat-gate barrier similar to those of the cohesion experiments. Each screen had a 5×5 cm. gate in the lower right-hand corner and was placed parallel to and 10 cm. from the front of the tank. The fishes were allowed four days of acclimation with the gates open and one day with them closed. They were then trained through 29 daily trials to come through the gates for food. Characteristic learning curves resulted in which the groups of four excelled the isolated fishes as in earlier experiments. Then, just after the twenty-ninth trial the fishes in two of the four group tanks were isolated into individual tanks; the other two groups remained intact. On the other hand, eight of the fishes that had been trained isolated were gathered into two groups of four each in two tanks; the other eight isolated fishes remained as before. All fishes remained in the same type of screen-barrier tanks as those in which they had been trained. Merely the grouping arrangement had been changed. Every fish in every tank was picked up with a net whether it was shifted to a new tank or not. The fishes were then given a four weeks' vacation from visual stimuli, trials, and feeding. At the end of this time they were given three daily trials. Seven fishes died during the retention period, so very little reliance can be placed on the data obtained. The results showed no certain influence of the group on retention and also showed very poor retention, particularly on the part of the grouped fishes.

Experiment 17.—This experiment was identical with Experiment 16 except for these changes: the fishes were allowed a ten-day acclimation period and a two weeks' vacation from trials and feeding just after the tenth trial. Their original grouping was retained throughout the experiment; no shift was made either before or after retention. Apparently the retention period was too short to test for an outstanding difference between group and individual retentiveness; none is discernible. Both the isolated fishes and the group retained their conditioned behavior perfectly after the two weeks' gap. In the preceding experiment with the four weeks' gap almost without exception the fishes performed less well after the retention period than they did on the very first trial of the experiment.

Experiment 18.—Twenty-four goldfishes were placed into sixteen aquaria with flat-gate screens such as those used in the previous experiment. Three fishes were placed in each of tanks 4, 8, 12, and 16. All other tanks contained one fish each. After an eight-hour acclimation period behind closed gates normal feeding trials were begun. After the fifth daily trial the fishes were "fed" a small bit of red rubber tubing about the size of the usual bit of fresh worm to which they had become accustomed. There was no retention period. The rubber reward continued for eleven daily trials. The results of this experiment showed indications of better retention by the fishes in groups than by the isolated fishes but not significant probabilities of better group retention.

RETENTION IN SPITE OF PUNISHMENT

Experiment 19.—This was a continuation of Experiment 5. Only one modification was introduced: from the eleventh trials onward the fishes were "fed" bits of freshly cut worm that had been soaked in glacial acetic acid just before the feeding. Other conditions were the same. At first the fishes persisted in biting the acid meat upon performing the cone-gate maze. Their violent expulsive mouth movements showed however that it was no reward. They gradually lost their avidity for the doctored meat and some of them eventually refused to come through the gate in the allotted half-hour of presentation time. If a fish did not respond within half an hour the gate and tank were closed until the next daily trial and that fish arbitrarily credited with a reaction time of half an hour even though it would have taken more time than that. This had to be done because in this experiment, time was not available for indefinite waiting. Many fishes persisted in coming through the gate for a long time after they refused to bite or even swim toward the acetic worm. Fishes that did not react within half an hour on three consecutive days were ignored in further trials except to be credited with a half-hour reaction from then until the end of the experiment. This was done because a fish that would not react for three days might lose its conditioning against the punishment, as several apparently did, and begin coming through again. Seven of the thirty-two fishes died during the retention part of the experiment and their records were

incorporated in the final reckoning only for those periods during which they seemed to be in good health. The results of this experiment show that all of the groups of fishes—twos, fours, and eights—retained conditioning in the face of punishment better than did the isolated fishes. The results seem to bear out those of Experiment 18 on retention through time. It is quite possible, however, that the rise in the curve of the isolated fishes after the application of acetic

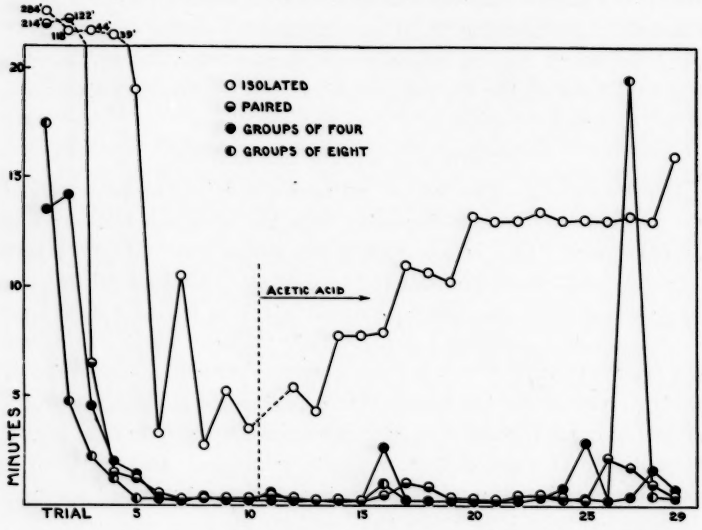

FIG. 6.—Mean reaction times of goldfishes on successive punishing trials. Experiment 19.

acid is in some way connected with their slower original conditioning. The peak in the curve of the group of eight on the twenty-seventh trial (Fig. 6) is unaccountable. All the fishes remained huddled in one corner of the tank for ten minutes after the gate was opened, then they slowly and cautiously began to swim about. All six of them (two had died) came through within two minutes of one another.

Experiment 20.—In the same way, this was a continuation of Experiment 17. After the twenty-eighth trial of the original experi-

ment worms were dipped in glacial acetic acid before being offered the performing fishes. Since 11 of the 32 fishes died by this time the results are not of much significance. Curves of isolated and grouped fishes crossed frequently and probably indicated erratic behavior on the part of both. The results do not corroborate those of the previous experiment. Data are not given.

Experiment 21.—Taking advantage of the fact that fishes have to learn to take food from the feeding needle, an effort was made to see whether groups influenced the retention of this conditioned behavior. Eighteen 45 mm. goldfishes were distributed in 12 small tanks: three fishes each in tanks 4, 8, and 12; one each in all the other tanks. The tanks were shielded from one another with black paper, filled with 15 cm. of water, and contained no screens. As usual the stimulus was the opening of the front cardboard which let in additional daylight. The fishes were conditioned to eat the bit of worm from off the needle near the surface of the water. After 21 such trials each fish was presented with a bit of worm that had been dipped in acetic acid. This "food" was placed approximately two inches in front of each fish so that it could not avoid seeing it. Every fish in the experiment was offered 60 such pseudo-feedings: 30 on the first day of acid trials, and 10 on each of the three following days. The reactions to the acid food varied all the way from seizing it to avoiding it by swimming away. By assigning arbitrary numerical values to each type of response a quantitative estimate of retention of the feeding habit was arrived at. There was no significant difference between isolated and grouped fishes in the first ten acid trials, but in both the last ten acid trials and in the total performance for the 60 acid trials the groups retained their conditioning about 11 per cent better than did the isolated fishes.

EXPERIMENTS IN MOTIVATION

In the foregoing experiments the food reward served as the motivating factor that stimulated learning. The group itself seemed also to provide a motivation that the isolated fishes lacked. The experiments in this section form an inquiry into the nature of these two moving factors and some of their interrelationships.

EXPERIMENTS IN FOOD DRIVE

Experiment 22.—This was a continuation of Experiment 13 under the section on Group Cohesion. From the twelfth to the thirty-seventh daily trials the fishes showed no appreciable increase in learning as revealed by their mean reaction time. On the thirty-eighth day the fishes in all tanks were given seven trials in as rapid succession as possible with normal feeding at each trial. The first tank was given one trial, and then the second tank one, then the third tank, and so on. As soon as the last (eighth) tank in the row had had its trial, number one was again opened, the fishes stimulated, and fed when they reacted. Then tank one was closed and tank two opened and run; and so seven times through the series of tanks. Then, after skipping a day, the same fishes were given eight trials in rapid succession but this time with no feeding. As a check on the results obtained, the fishes were given a day's vacation and then tested again, twice in succession with feeding and on the next day twice in succession without feeding. Results: on successive feeding trials the fishes reacted more and more slowly until, during the later trials, they became very sluggish and seemed to perform only by virtue of random movements. Toward the end of the feeding trials several fishes failed to take the offered bit of fresh worm. No appreciable difference appeared between the performances of the cohering and separating groups. The reaction time of the first of the consecutive no-feeding trials was about the same as that of an average normal trial. But the seven subsequent no-feeding trials proved to be the quickest and most consistent of the entire experiment. The check trials made two and three days later showed the same general results. The curves for this experiment are shown in Figure 7.

Experiment 23.—The same technique was used to determine the effect of feeding- and non-feeding trials in a continuation of Experiment 17 after the 15th trial. Of the original 32 fishes, two isolated ones proved refractory and did not respond within half an hour, two other isolated fishes had died, and two from the groups had died. These fishes were not involved in this experiment. Six consecutive feeding trials and six consecutive trials without feeding in general repeated the results of the foregoing experiment. The "best" performance of the 36 trials of the whole experiment came during the non-

feeding trials; the "worst," during the consecutive feeding trials. No differential effect on the groups and isolated fishes was evident. Data are omitted for lack of space.

EXPERIMENTS IN FOOD CONSUMPTION

Experiment 24.—A preliminary experiment was undertaken to see whether the group affected the food consumption of goldfishes. Medium-sized earthworms were cut into bits about 2 mm. long by means of a set of 12 parallel mounted razor blades. These bits were thoroughly rinsed in tap water and then fed to medium-sized goldfishes distributed singly, in pairs, and in groups of four in aquaria that each contained the same amount of water. The pieces of worms

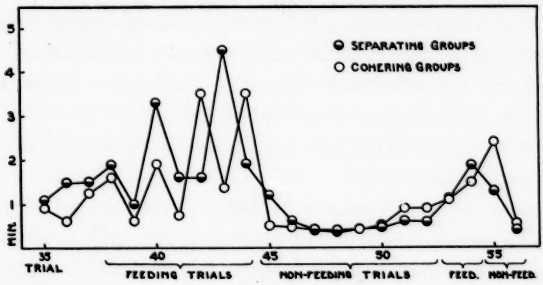

FIG. 7.—The effect of giving and withholding reward in successive trials with goldfishes. Mean reaction time. Experiment 22.

were counted when they were placed in each tank and the uneaten surplus counted when removed at the end of an allotted feeding period of one-half hour. Even though the clitellum and two ends of each worm were not included, it was impossible to insure equal-sized pieces—a thing most necessary for determining quantitative food consumption. This fact coupled with early deaths among the fishes invalidated what results there may have been. All that can be said is that there was a rough indication that the individuals in the groups ate more per fish than did the isolated fishes.

Experiment 25.—Since earthworms did not prove satisfactory as a measurable food, *Daphnia* were substituted and a smaller species of fish used to cut down consumption. The lake shiner *Notropis atherinoides* served very well for this experiment provided it was kept

cool. Each of seven small aquaria used stood in cool running water on a water-table; aeration was provided by bubbling air. Four shiners averaging 46 mm. in length were placed in one tank holding 8 liters of water; four fishes averaging 45 mm. were placed two each in two tanks of water that each held 4 liters of water; four fishes averaging 46 mm. in length were placed singly in four aquaria that each held 2 liters of water. All tanks were visually isolated from each other by black-paper wrappings. The *Daphnia* used for feeding were sifted so that only the larger sizes were retained. Into each tank was placed at each feeding a greater number of counted *Daphnia* than the fishes in it could consume in an hour. At the end of an hour's feeding the surplus was removed and counted, the difference between the two counts being the number of crustaceans eaten by the fish or fishes in a tank. The result of six days' feeding, one hour each day, revealed that the most food was consumed among the fishes in the group of four. Each fish in the group ate on the average twice as much as the average fish among either the isolated or the paired fishes.

Experiment 26.—Thirty-one paradise fishes, *Macropodus opercularis*, were accurately measured by means of what were essentially photographic contact profile silhouettes on bromide enlarging paper. Of these fishes twenty-four were selected because of their similar sizes and were distributed in glass crystallizing dishes thus: four fishes each were placed in two vessels containing 5 liters of water each; two fishes each in four vessels containing $2\frac{1}{2}$ liters of water each; one fish each in eight vessels each holding $1\frac{1}{4}$ liters of water. The fishes in the groups of four averaged 28.0 mm. body length; those that were paired, 27.8 mm.; the isolated fishes, 27.8 mm. Black paper prevented extraneous visual stimuli and cardboard tops prevented the fishes from jumping out of the shallow dishes and foreign matter from dropping in.

Live *Daphnia* too large to pass through a wire screen 16 meshes to the inch were used for daily feedings. They were counted out into small beakers with a mouth-controlled pipette and then poured into a given vessel holding the fishes. At the end of exactly an hour the *Daphnia* remaining in each tank were removed with the pipette,

counted, and discarded. Feces were also removed by siphoning and the water-level was restored. The difference between the two counts for a given tank revealed the number eaten. Tanks were fed in rotation and the direction was reversed daily: one to fourteen one day, fourteen to one the next. The experiment continued through 33 daily feedings. In ten instances feedings for one day, and in two instances feedings for two consecutive days, had to be omitted to allow the supply of *Daphnia* to catch up with the fishes' appetites. The results show rather clearly that being in a group increases a fish's food consumption. For the first 22 feedings the fishes in the groups of four ate more sifted *Daphnia* per fish than did the isolated fishes on the average, and they exceeded the consumption of the paired fishes every day of the experiment but the third. From the twenty-third day on the food consumption of the isolated fishes and that of the members of the groups of four was about the same. Very few dead crustaceans were removed from the feeding vessels; the fishes usually ate all that they attacked. Where dead ones were left they were counted as live animals if they were whole; otherwise they were estimated as fractions of one animal.

Experiment 27.—Twenty-four zebra fishes, *Brachydanio rerio*, were measured in a narrow glass cell with a metric scale and then distributed in 14 crystallizing dishes as above. The two groups of four were in 2,600 cc. of water each, the four groups of two in 1,300 cc. each, the eight isolated fishes in 650 cc. each. Other conditions matched those of the preceding experiment. The *Daphnia* used were such as would pass through a 16-mesh-per-inch screen but not through an 18-mesh screen. The fishes were allowed to feed an hour. After the twelfth consecutive day of feeding the isolated fishes were gathered into groups of four and the groups of four were isolated into eight vessels. The original relation between size of group and amount of water was retained until the eighteenth feeding; from then on each tank contained the same amount of water—2,600 cc. The fishes were shifted back to their original groupings after the twenty-third feeding. Between the sixteenth and seventeenth feedings there was a three-day gap to allow *Daphnia* to multiply. Four fishes died during the experiment; all of the fishes were rather thin at the beginning.

The results (Fig. 8) show that grouping the fishes together undoubtedly increased their food consumption. Each day the isolated fishes averaged fewer *Daphnia* per fish than did the fishes in the groups of four. The eating of the paired fishes was rather irregular, starting in at first between the other two types of groupings and ending above both in quantity consumed. Equalizing the amounts of water in each vessel seemed to increase the food consumption of the isolated fishes more than it did that of the pairs or groups. Shifting groups showed conclusively that differential consumption

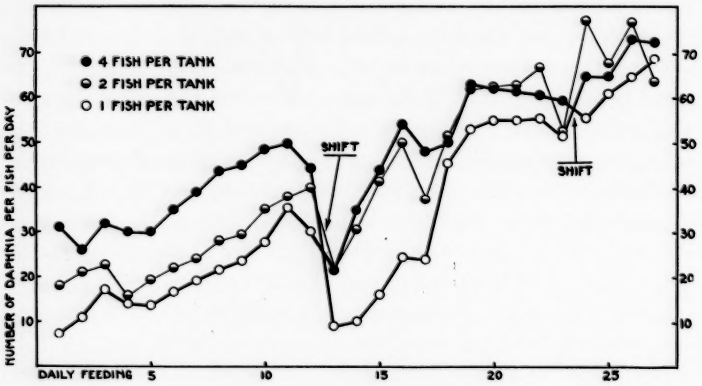

FIG. 8.—The effect of grouping on food consumption in zebra fish

did not depend on inherent individual appetite or size of fish but primarily on grouping.

Experiment 28.—Sixteen goldfishes of about 37 mm. body length were accurately measured by means of photographs of equal magnification and then placed in ten crystallizing dishes—four each in two of them and one each in the remaining eight. There were two litres of water in each dish. The fishes were nearly balanced as to length: the isolated fishes averaged 36.97 mm. body length and the grouped fishes 37.10 mm. A shift in the grouping later gave this slight advantage in size to the isolated fishes for the significant part of the experiment. The fishes were fed for one hour daily with all the large *Daphnia* (too large to pass a 16-mesh screen) they would eat. Rather than not feed the fish when the supply of *Daphnia* ran low, a sub-

stitute non-measurable diet was used. It was composed of one-half part dry rolled oats and the other half a prepared laboratory fish food containing dried shrimp, calves liver, eggs, flour, baking powder, ordinary salt, and magnesium sulphate. The fishes were fed this food on these days of the experiment: 17, 18, 19, 21, 22, 24, 25, 27. At the end of each hour's feeding the surplus was siphoned off. After three days of feeding the groupings were shifted: the isolated fishes were placed into groups of four per vessel and the grouped fishes were isolated. The results (Fig. 9) verify those of preceding experiments. The fishes of the groups of four consumed more

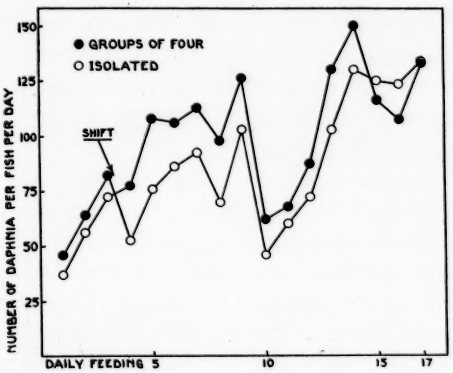

FIG. 9.—The effect of grouping on goldfish eating. Experiment 28

Daphnia every feeding except the last three. A possible explanation of this inversion in consumption at the end of the experiment is that the *Daphnia* became so numerous in the tanks of four that they presented too many stimuli; the fishes were observed to take after an isolated crustacean and eat it whereas a dozen or so in its immediate field of vision seemed to offer conflicting stimuli that blocked the feeding response. Working on such a hypothesis the experimenter tested the two groups thus on the last day: one group of four was given its usual quota of *Daphnia* all at once in the customary manner, the other group was given only a hundred at a time. As it exhausted these another hundred was poured into the tank, and so on. This prevented the *Daphnia* from becoming too concentrated in the

latter vessel, especially during the early part of the feeding hour. The isolated fishes were fed as usual. Whereas the control group (fed in the same manner each day) ate 64 more *Daphnia* this day than it had on the previous day, the experimental group ate 140 more on its second day. As in the foregoing experiments, the shift in grouping shows conclusively that the differential consumption is a matter of grouping and not one of inherent size of appetite. Precise measurements of the fishes showed that although the members of the groups ate more than did the isolated fishes, they grew significantly less.

ON THE EFFECT OF SEEING OTHER FISHES PERFORM A MAZE

Observations made during many of the above experiments tended to substantiate an explanation of group cohesion based largely on an interplay of visual stimuli. An experimental attempt was made to isolate and evaluate this factor by testing whether allowing fishes to watch other trained fishes run a maze influenced their ability to master it afterward. That is, is there a tendency toward imitation in fishes? The apparatus used (Fig. 10) consisted of large aquaria divided into two chambers, one narrow and one wide, by a glass partition extending from the front to the rear of each tank and from the bottom approximately to the top. The narrow compartment was 6.5 cm. wide and the wider one 9.5 cm. In four of the tanks this partition was painted black and weathered (to remove oils), in the other four it was left clear. Ten cm. from the front end of the larger chamber was placed a typical flat-gate screen barrier with a 5 cm. opening at the lower right-hand corner against the glass partition. Heavy airplane celluloid sliding doors were used in place of the less smoothly operating metal ones. The experiment consisted in allowing untrained fishes in the smaller chamber to see trained fishes perform in the larger one on the other side of the partition, and then, after removing the trained fishes, placing the untrained fishes in the larger chamber to see if they profited by their observations. The tanks with opaque glass were controls. Trials were made in daylight.

Experiment 29.—Four 45 mm. goldfishes were placed in the large chamber of each of the eight tanks. The two types of tanks were arranged alternately in a row. After 11 days of training the 32 fishes averaged 22 seconds reaction time. Unselected, untrained 45 mm.

goldfishes were then placed, one each, in the smaller chamber of each tank. Only in tanks 1, 3, 5, and 7 could they see through the glass partition. One hour after the new fishes had been introduced the usual trials with the trained fishes were resumed with this difference: when they came through the gate they were not fed. Otherwise they were treated exactly as they had been during the training

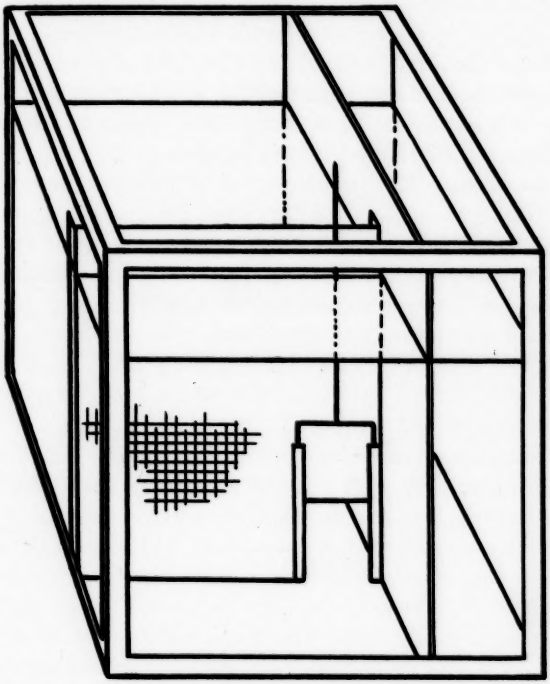

FIG. 10.—Type of aquarium used in Experiments 29 to 33

period. Ten such no-feeding trials were run in the next four days; then these trained fishes were removed and the untrained fish in each tank was shifted from the smaller chamber into the just vacated larger chamber. Four hours after this shift the new fishes were given their first trials. The cardboard fronts were removed, the gates opened, and the fishes fed bits of worm from the feeding bristle upon successful performance. Trials continued three days only. On the

first day the fishes in the clear-glass tanks (that had been able to see the trained fishes perform) reacted with a mean time of 30.80 minutes, on the second day 4.74 minutes, and on the third day 4.59 minutes. The fishes in the opaque-glass tanks responded less rapidly: on the first day the three fishes in tanks 2, 4, and 6 together averaged a reaction time of 102.00 minutes while the fish in tank 8 did not respond in over 4 hours. On the second day all these fishes did not come through in over 80 minutes of stimulus presentation. On the third day the fish in tank 4 came through in 8.98 minutes but the other three fishes did not come through in over 80 minutes. The results are at least indicative of faster conditioning among the fishes that could see their trained fellows perform.

Experiment 30.—The trained fishes of the previous experiment were used as pace-setters for this one. After these trained fishes were again conditioned so that they responded in less than one minute (mean reaction time: those in clear tanks—29.0 seconds; those in opaque tanks—37.2 seconds) *three* new untrained fishes were placed in the small compartment of each tank. After four hours' acclimation first trials were run. The trained fishes reacted as before, only they were not fed. All tanks were given one trial the first day, seven the second, and two on the third day—ten in all. A slight slowing down of the reaction time of the trained fishes occurred, possibly because they were not fed when they responded. On the last trial, however, their mean reaction time was less than a minute. Then they were removed from the tanks and the untrained fishes shifted from their small chambers into the vacant large ones as before. An hour later they were given their first trials. They were fed as they responded successfully. The mean reaction times of the two types of groups shows a slight advantage for conditioning correlated with having lived in the clear-glass aquaria. See Figure 11.

Experiment 31.—This was a repetition of the above experiment with two changes. Twenty "pre-conditionings" were given the untrained fishes, i.e., those trials during which they observed (or not, in the case of fishes in the opaque tanks) their trained fellows through the glass partitions. The trained pace-setters were fed upon every successful response; this probably prevented the slowing down of the reaction time of the trained fishes. The mean reaction time for their last five pre-conditioning trials was roughly 15 seconds. An

attempt was made to prevent differential stimuli resulting from varying presentation time by keeping open the opaque-glass

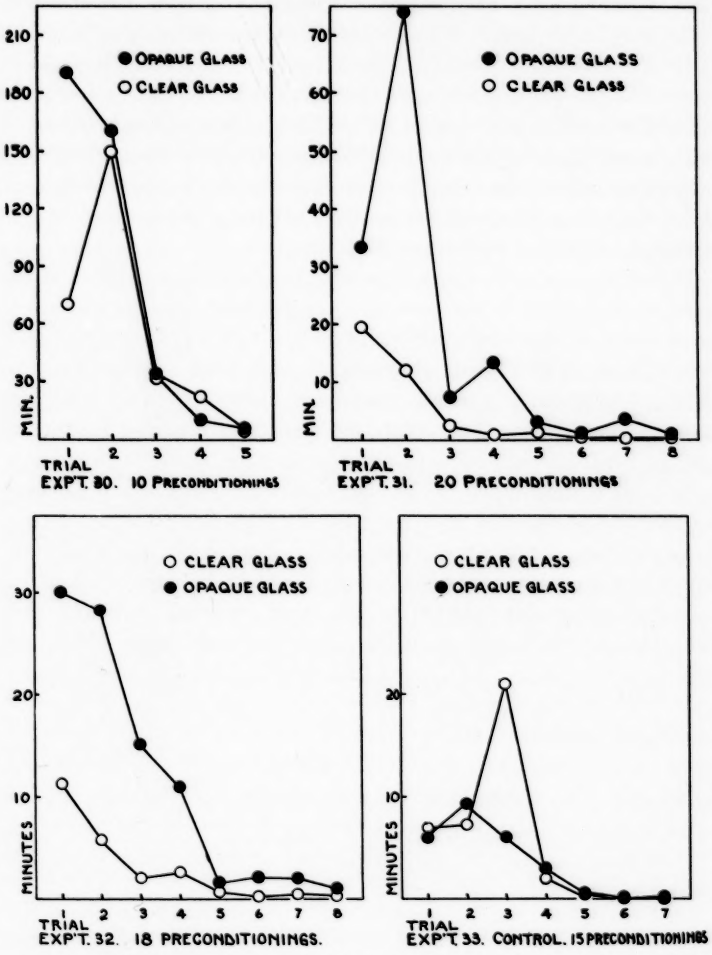

FIG. 11.—Mean reaction times of goldfishes in Experiments 30 to 33 on the effect of seeing other fishes perform a maze.

aquaria only as long as their paired clear-glass tanks. Since the trained fishes in both types of tanks responded in so nearly the same

time no great effort was necessary to insure equal lengths of presentation time. An hour after the trained fishes had been removed and the untrained fishes shifted into the larger chambers, the first trial was given to the latter. Trials were given once on each of the seven following days. The results (Fig. 11) show a clear distinction between the two types of groups: the fishes from the clear-glass tanks definitely reacted more quickly than their opaque-glass fellows.

Experiment 32.—This was a duplicate of the preceding experiment with one modification; the three untrained fishes in each tank were given 18 pre-conditionings before being shifted. The results (Fig. 11) strikingly corroborate those of Experiment 31.

Experiment 33.—To check a possible conditioning influence of the type of tank itself in the preceding experiments, a control experiment was run with new untrained fishes as follows: three goldfishes were placed in the small chamber of each tank. There were no trained leaders in the larger chambers. However, trials were run just as if there were. The cardboard front was removed from each tank in turn, its gate opened, and the feeding needle with a bit of freshly cut worm dipped in the water three times as if to feed three successively performing imaginary fishes. Then after a lapse of time equal to that of the usual trial, about 30 seconds, the tank was closed. This routine was repeated 15 times in four days; then the untrained fishes were shifted to the large chamber as before and given feeding trials just as in the previous experiments. Probably because of the hot weather, four of the twenty-four fishes died during trials. The records for tank 3 (clear glass) were deleted because of deaths and erratic behavior. Were the data for this tank included in the general summary they would not change the significance of the results. The results (Fig. 11) show clearly that the difference in behavior between the two types of groups of fishes in the preceding experiments cannot be directly attributed to the nature of the types of mazes used.

SOURCES OF DIFFICULTY AND ERROR

Many disturbing and complicating factors entered into the above experiments. For one thing, excepting *Fundulus*, fishes were selected only for health and size. Sex could not be told by external examina-

tion of such small fishes. The fishes did show signs of sex behavior such as persistent chasing of each other. Age, although not controlled, was largely eliminated as a variable through selection of size. Conditioning previous to purchase, probably unimportant, could not be controlled as the fishes were procured from a wholesale dealer.

Numerous disturbing stimuli were common. Rumbling trucks on a nearby street, slamming doors, passing people, all caused fright reactions in the fishes at times. Temperature and light were but roughly controlled. Extremely hot weather was usually accompanied with high mortality; epidemics of fluke parasites, *Gyrodactylus*, and dropsy killed others. Inherent variations were undoubtedly present in their behavior; the refractory fishes may have represented an extreme variation.

An obvious difficulty arose from the habit common among goldfishes of spitting out food and seizing it again. Sometimes after a given fish had performed and was fed, it would swim back into the original chamber from which it had come and then spit out the worm. Now and then another fish that had not yet performed would seize the food and eat it. Both fishes would be disturbed in their behavior by the experience. As another source of error the fishes showed evidence of becoming conditioned against a closed gate so that when it was opened for trials they would stick their noses half an inch through the opening and come no farther; sometimes they remained in such a position for hours.

One factor that introduced a differential stimulus resulted from multiple feedings in group tanks. The first fish to perform was always fed immediately, and quite often, if not usually, the fishes that had not yet performed saw this fish get fed. Naturally this stimulated them. That it resulted in speeding up the reaction time of the groups is an unwarranted conclusion. Observations showed that this added stimulus more commonly than not blocked the usual and more intelligent response by stimulating the fishes to a blind effort to penetrate the screen. Another variable resulted from the size of the screen opening in relation to the size of the fishes: it was such that ordinarily the fishes in groups could not come through simultaneously. This fact adds further significance to the results showing quicker

response in the groups because the restriction offered groups, but not to isolated fishes, tended to inhibit their reaction.

A somewhat subtle variable entered with the conditioning of the experimenter. In general it seems to him likely that the greater this conditioning, the more reliable the results obtained.

Another greatly disturbing factor resided in the unnaturalness of the conditions under which the fishes lived. The tanks were necessarily artificial, cramped, and angular quarters.

DISCUSSION AND CONCLUSIONS

In spite of difficulties and unknowns the experiments were performed with large enough numbers and repeated often enough that one can feel reasonably safe in drawing certain conclusions.

Groups of goldfishes in a simple maze become conditioned to a simple motor response pattern more quickly than do comparable isolated fishes. Contrary results with mudminnows on a different problem caution against generalizing.

Although group cohesion is seen to be a factor in the quicker learning of the group it is difficult to see in what way it tends to shorten reaction time. It is true that fishes reacting rapidly if isolated did no more than reduce the arithmetical mean time of reaction for all isolated fishes, whereas, if grouped, they not only had this statistical effect but exerted a physiological influence on their fellows as well. On the other hand, if this factor acts merely as a matter of group cohesion, the groups would tend to hold back the pioneers whom they later would follow. This, in fact, is what happened. Detailed data for Experiments 2 to 5 show that for the first two days the fish which gave the most rapid reaction in every experiment was found among the isolated fishes and not in the groups. Likewise, the very slowest fish to respond was found among the isolated fishes. This shows a greater variation in reaction time among the isolated fishes. Very commonly all four or eight fishes in a group will have reaction times (on a given day) not differing more than two or three seconds; very rarely did such concerted behavior appear among comparable isolated fishes. Cohesion, then, explains the more or less united response of a group but it does not explain the shorter mean reaction time of the groups.

Probably the accelerated condition of the group depends on cohesion plus two other important group effects. Although it was not possible to make quantitative measurements, observations showed clearly that inter-stimulation between members of group kept the group active at times when many of the isolated fishes in the same experiment were inactive. The greater the activity of a given fish the greater its chances of finding the gate of a maze, particularly in the earlier trials. The other group effect that probably stimulated quicker reaction time was, paradoxically, a quieting effect. Fright reactions were more common among isolated fishes; the normal relatively quiet exploratory movements were more common among grouped fishes. Once a fish in a group finds its way through a gate, group cohesion tends to pull the other after it. And, of course, tends to pull the pioneer back to the group. Having fed, however, the pioneer is less active than it was immediately before performing the maze, so the chances favor its staying put and the group coming through to it.

It is impossible to say to what extent the dropping of the learning curves actually represents learning and to what extent it represents either increased activity due to anticipation of reward or to a gradual release from inhibitions set up by the experimental conditions. That this latter is an important factor seems to be revealed by the shorter reaction time of the untrained fishes of the "imitation" Experiments 31 and 32 following twenty pre-conditionings as compared with the untrained fishes of Experiment 30 after having had only ten pre-conditionings. The greater the number of pre-conditionings the greater the freedom of the fishes from inhibitions.

The leadership experiments showed conclusively that a trained fish influences the behavior of untrained fishes. It remains to be proven that this is more than a group-cohesion phenomenon; the lure-fish experiments provide mild indications that it is.

The split-group experiments were inconclusive in that the succeeding experiments failed to give consistent results. What little evidence there was agrees with the group-learning experiments in revealing a tendency toward cohesion which would be an advantage in conditioning behavior.

The fact that members of a group eat more food in a given time than do comparable isolated fishes seems to imply some competitive

factor. Observations such as those mentioned in the introduction concerning the feeding of baby chicks and angel fishes seem to bear out this inference. The immediate explanation that occurs is that the fishes in groups stimulate each other to greater activity and hence need more food for energy production. Quite commonly isolated fishes were quiescent during the feeding period while the fishes in the groups were actively swimming about. Work by Schuett (1933), verified by Bowen (1933), on the respiration of groups of goldfishes versus isolated fishes in oil-sealed Erlenmeyer flasks showed that the fishes in groups of four used only about one-half as much oxygen per fish as did the isolated fishes under the same conditions; the same relations held for fishes contained in flasks of slowly flowing water. There still remains the possibility that the fishes in the open crystallizing dishes of the feeding experiments respired differently from the fishes in the flasks. The rapidly increasing food consumption of all the fishes in the feeding experiments can be only partially accounted for by the slight increase in the size of the fishes. It probably indicates a gradual release from inhibitions set up by the experiment; the sudden drop in consumption upon any unusual disturbance such as a shift in grouping (see Fig. 8, thirteenth trial) bears this out. Further, the fishes possibly learn to eat more rapidly as they learn that the period of feeding is restricted.

Whether fishes imitate each other seems largely to be a matter of definition of the word. Fishes probably do imitate on a low or simple instinctive level. The differential food consumption just discussed may quite well be explained on a basis of instinctive imitation rather than competition. Seeing one fish eat may set off the feeding mechanism in another fish that would otherwise not have eaten just then. The results of Experiments 29 to 33 provide no grounds for ascribing inferential imitation to the fishes involved. The results can be explained on a simpler basis. The untrained fishes that could see through the clear-glass partitions and watch their fellows react to the stimulus probably became conditioned in two ways that the untrained fishes in the opaque-glass tanks did not. First, they became "reassured" by the reaction of the trained fishes that the stimulus was not followed by harmful results. This provided a quieting effect. Secondly, they learned, through visual cohesion with the

trained fishes, to move forward with them when the stimulus was presented. In Experiments 31 and 32 the untrained fishes were at times seen even to precede the trained fishes in moving toward the stimulus in their respective chambers. Very probably there was some transfer of both types of conditioning when the untrained fishes were shifted to the larger chambers. The fishes in the opaque-glass tanks had no opportunity to acquire either of these types of conditioning. They commonly moved to the rear of their chambers and remained there during each of the pre-conditioning trials.

The results of these experiments relate to schooling in several significant items. An attempt was made to determine whether goldfishes school by turning 26 of them loose in a pond about 50 feet in diameter. They scattered immediately and were never seen after that although efforts were made to find them at intervals during the two months that followed. In small concrete outdoor pools nine feet square goldfishes were observed to school at times and to be scattered at other times. In aquaria they tended to school when disturbed. If it should be generally true that fishes learn more readily in groups than when isolated, great survival value would attach to schooling behavior, especially among young fishes. Parr (1927) discovered that an isolated three-inch chub mackerel would join a school of seven-inch mackerels quite readily, but that an isolated seven-inch fish would not join a school of smaller fishes. One can easily read an educational significance into such behavior.

The survival value of imitating the behavior of the group, particularly the imitation of adult members by the young, depends on the inherent survival value of the behavior patterns of the adult group. In human society reforms and innovations are often more desirable than blind imitation. However, these desirable changes rarely have to do with biological necessities—with such things as eating, mating, sleeping. They are more concerned with religion, art, politics, economics: biological non-essentials. In fishes the behavior patterns are rigorously limited to biological necessities. Very probably the behavior repertory of a given species of fish has been refined through long ages of evolution into an exceedingly efficient mechanism for fitting that species into its environmental niche. Therefore, the sooner a fish acquires the non-instinctive behavior of the

group, probably the better are its chances for survival. To illustrate: disregarding the possibility that bottom-scavenging is *purely* instinctive, a young goldfish schooling with and to some extent imitating the feeding habits of its older fellows will obtain a survival advantage over a fish reared alone. It will learn a type of effective feeding for which its carp-mouth is eminently adapted before it could have evolved a successful individual technique. Be it ever so slight, the advantage would provide survival value. While the illustration is imaginary, the principle may be quite real. Schooling undoubtedly affords enhanced opportunity for such learning by precept. The group-feeding experiments disclose a mechanism that seems to have a quite obvious survival value. The fact that a fish will eat more food and eat it more quickly when in a group than when isolated probably in the long run aids survival. With the supply of food limited as it nearly always is, the species without this mechanism is the one that will first starve.

The food-drive experiments open up further interesting problems. One can easily see why fishes react more slowly when satiated with food, but it is less easy to understand why they should speed up their reactions so greatly on successive trials without feedings. Stimulus-summation offers a tentative explanation. More than relative hunger is involved because the fishes that have been deprived of food for three days do not react as quickly as they do on the third successive non-feeding trial after only the usual one-day deprivation.

The experiments on retention are inconclusive in regard to differential effects of grouping. They need to be repeated under better conditions for survival and non-disturbance. It seems quite likely that there is within the group a mechanism that enhances retention. One fish, for example, that remembers a maze after a lapse of a month may conceivably stimulate others to perform it successfully. On the other hand, a priori, an opposite effect seems just as possible. Hunter (1929) summarizing experimental work in human retention shows that retention depends in part upon the type of activity undertaken during the retention period. Subjects merely resting after learning a set of nonsense syllables remembered them better than did subjects studying something else between learning and recall. The nervous stimulation of group activitity in fishes may conceiv-

ably prove to be a retention-inhibiting factor analogous to such interspersed mental activity in man. Here is an interesting field for further exploration.

That the group should influence the behavior of its members is to be expected, particularly so in the light of numerous experiments in the field of animal aggregations. Uvarov, whose work has been verified by Faure (1932), finds that crowding affects the structure and color phase of migratory locusts; Pearl and Surface find that egg-production in hens increases inversely with the size of the flock, and attribute this effect to a psychological factor; Reinhard and others have shown that crowding in aphids indirectly influences wing-production; Banta and Brown and Stuart show that sex in *Cladocera* is likewise influenced by crowding, the more crowded females producing the higher percentage of males. If morphology and physiology can be influenced to such an extent by the crowd, surely there are good reasons for expecting a crowd-influence on the behavior of animals, which after all is a species of physiology. For an exhaustive summary of the effects of aggregation on morphology and physiology, and for references to the instances just mentioned, see Allee's (1931) *Animal Aggregations*.

There are several logical channels into which this present inquiry might lead. The problem of the influence of numbers on appetite and food consumption has been mentioned. Another broader problem might deal with the nature and significance of the group influence on an unconscious level. A study of the effects of group size should be applied to animals as Hudelson and others have applied it in the field of human education. A more complete understanding of the function of childhood might contribute greatly to solving problems of group organization and education. Groos (1911) points out that play in the young of animals affords practice and exercise which provide requisite skill and strength for later independent life. The important facts seem to be that play is usually restricted to young animals and is *social*. A phylogeny of play—the evolutionary history of the relationship of youthful activity to adult group behavior—needs to be investigated.

The present investigation has shown that the group is a force in shaping the behavior of its members even when the group is com-

posed of animals low in the vertebrate scale. It has demonstrated that ends providing survival value may be gained through a group influence.

SUMMARY

1. Under certain experimental conditions mudminnows in groups exhibit an antagonistic behavior that inhibits learning.

2. Goldfishes become conditioned to run a simple maze more rapidly when grouped than when isolated.

3. A trained goldfish in a maze with an untrained fish will speed up the conditioning of the latter.

4. The group seems to stimulate learning behavior in three related ways: through group cohesion visually integrated; through an inter-"reassurance" that tends to remove environmentally induced inhibitions; through an interaction between members of the group that stimulates exploratory activity.

5. A goldfish learns to run a simple maze more rapidly after having seen other goldfishes run it.

6. There are indications that groups of goldfishes retain conditioned motor responses better than do isolated fishes.

7. A limited number of successive no-reward trials greatly stimulates conditioned responses in both grouped and isolated fishes.

8. Successive reward trials slow down conditioned responses in goldfishes, grouped or isolated.

9. Goldfishes, paradise fishes, zebra fishes and shiners eat more food per fish in groups than when isolated.

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